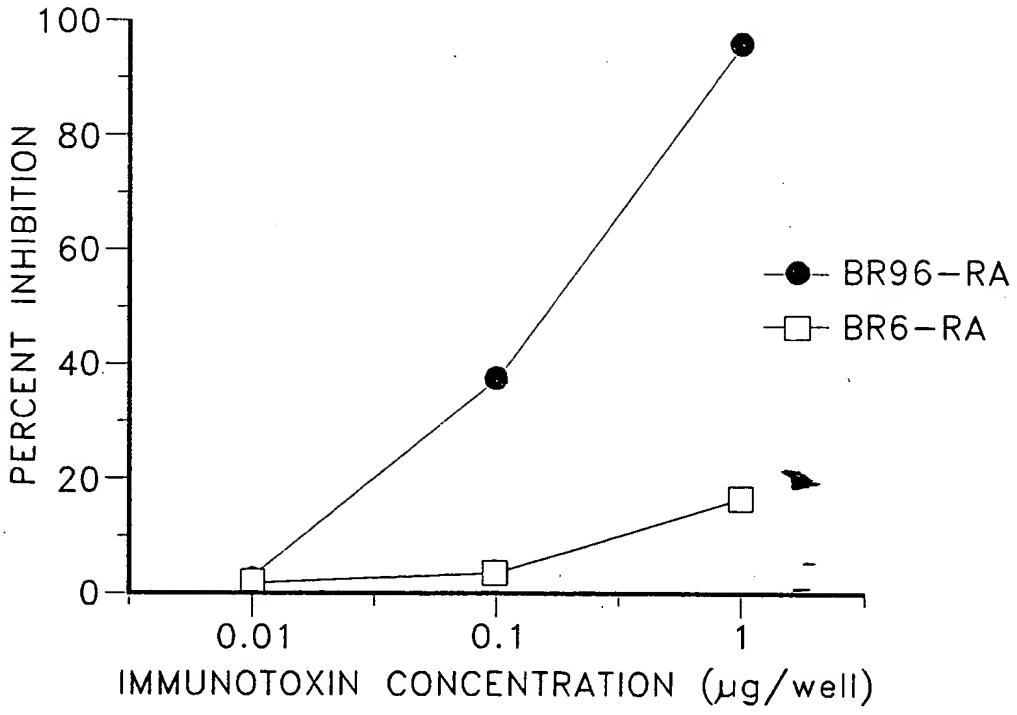

100
80
60
40
20
0
PERCENT INHIBITION
0.01
0.1
1
IMMUNOTOXIN CONCENTRATION (μg/well)
BR96-RA
BR6-RA

FIG. 1

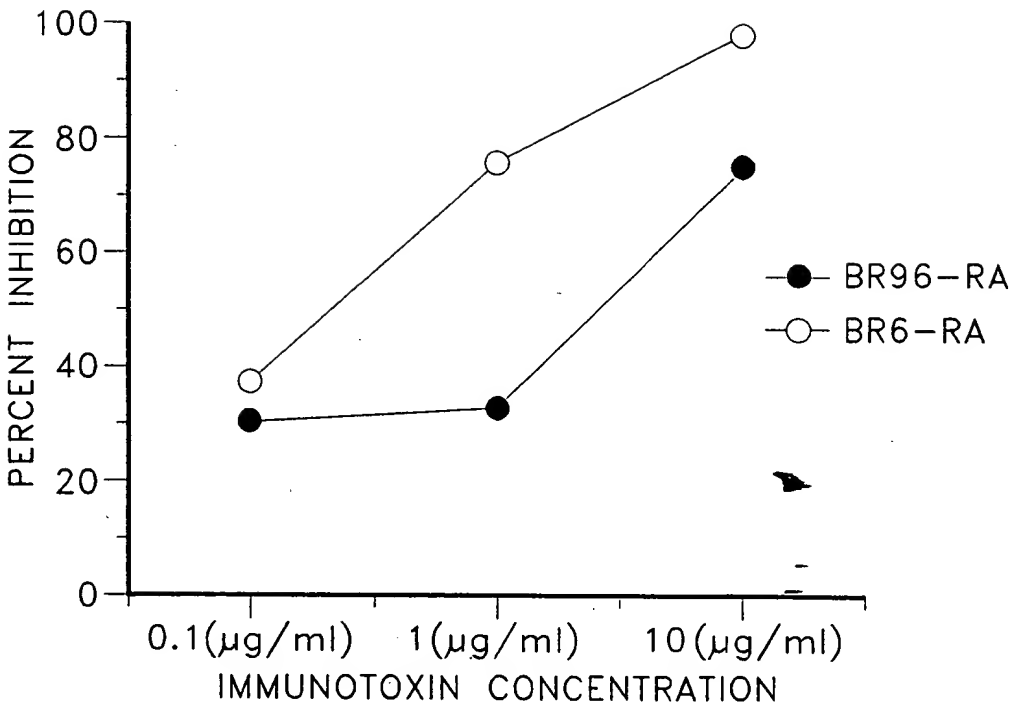

100
80
60
40
20
0
PERCENT INHIBITION
BR96-RA
BR6-RA
0.1(μg/ml)
1(μg/ml)
10(μg/ml)
IMMUNOTOXIN CONCENTRATION

FIG. 2

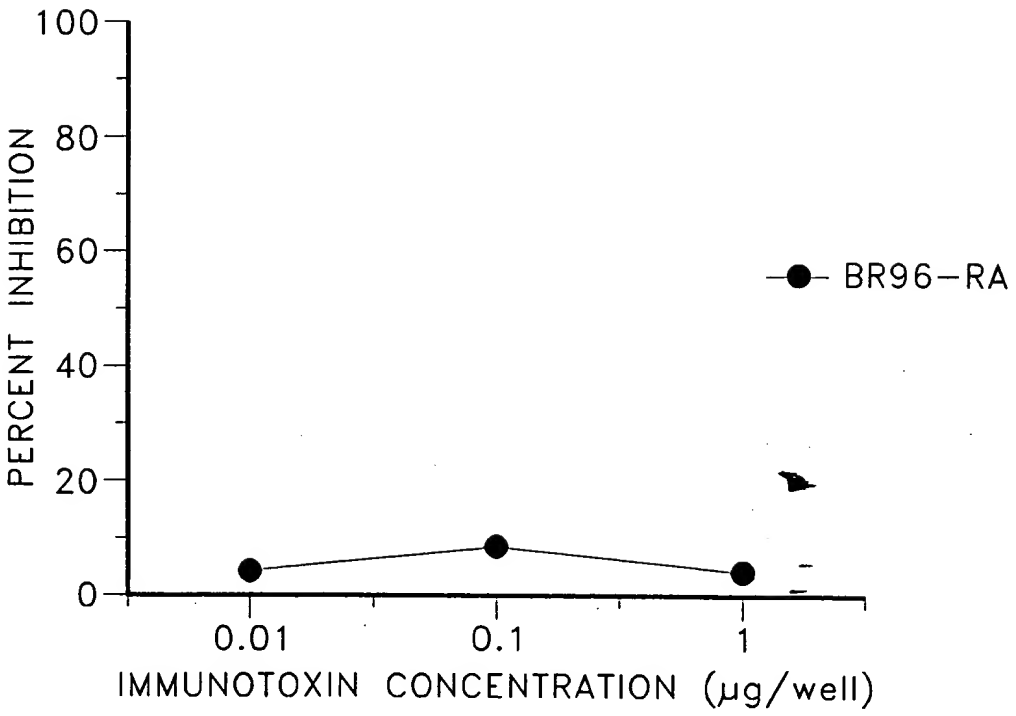

100
80
60
40
20
0
PERCENT INHIBITION
BR96–RA
0.01
0.1
1
IMMUNOTOXIN CONCENTRATION (μg/well)

FIG. 3

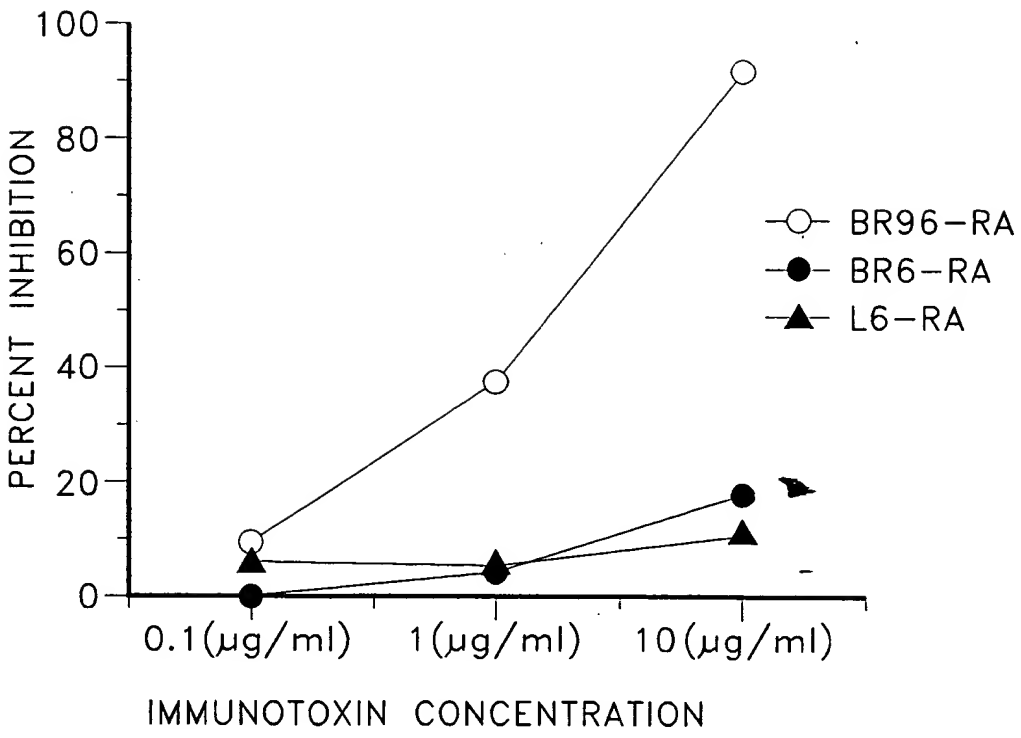

100
80
60
40
20
0
PERCENT INHIBITION
BR96-RA
BR6-RA
L6-RA
0.1(μg/ml)
1(μg/ml)
10(μg/ml)
IMMUNOTOXIN CONCENTRATION

FIG. 4

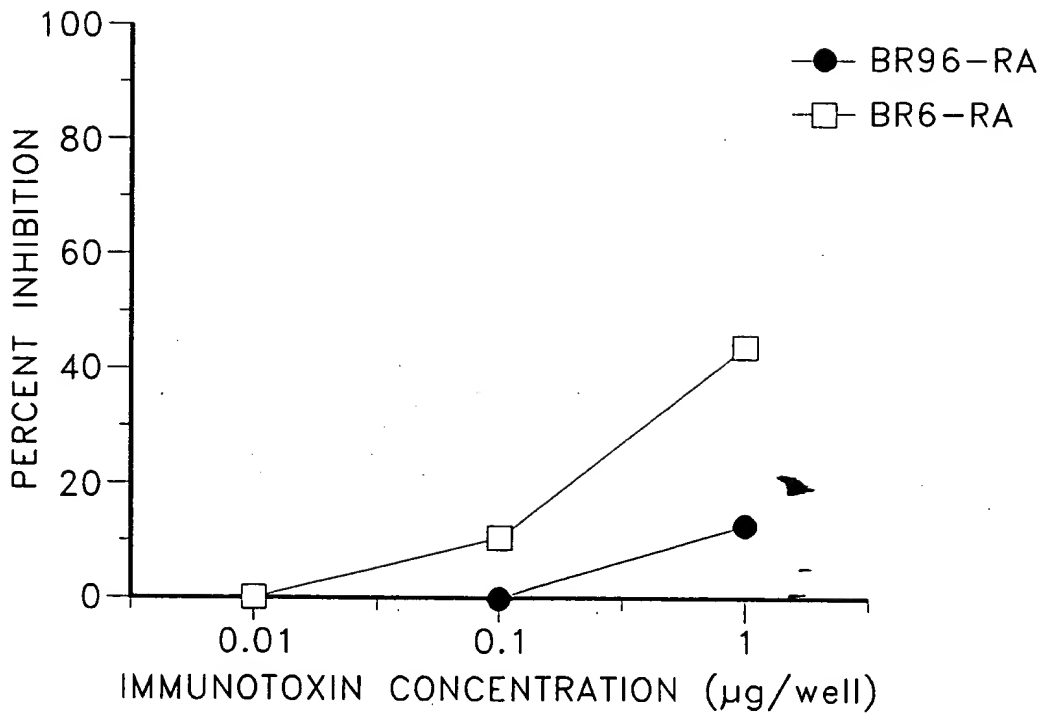

100
80
60
40
20
0
PERCENT INHIBITION
0.01
0.1
1
IMMUNOTOXIN CONCENTRATION (μg/well)
BR96-RA
BR6-RA

FIG. 5

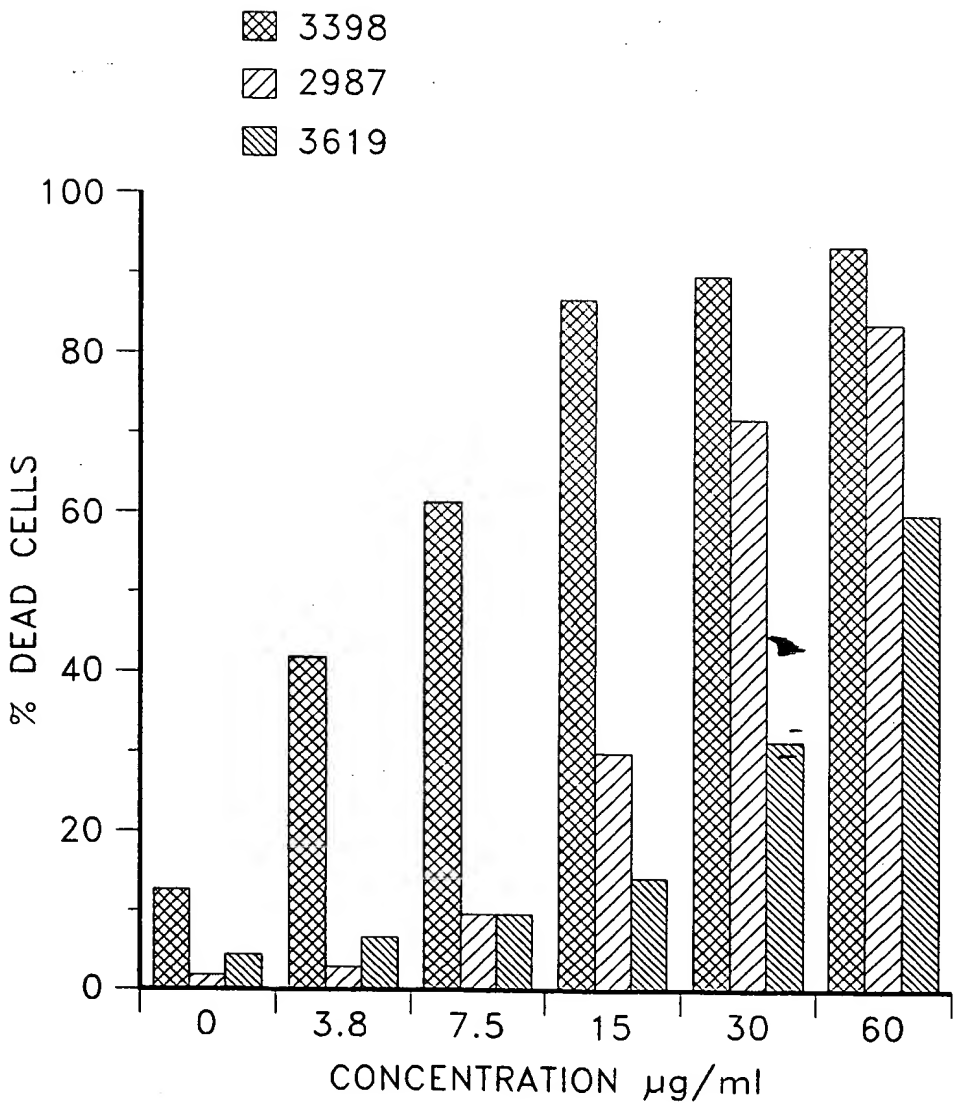

3398
2987
3619
100
80
60
40
20
0
% DEAD CELLS
0
3.8
7.5
15
30
60
CONCENTRATION µg/ml

FIG. 6

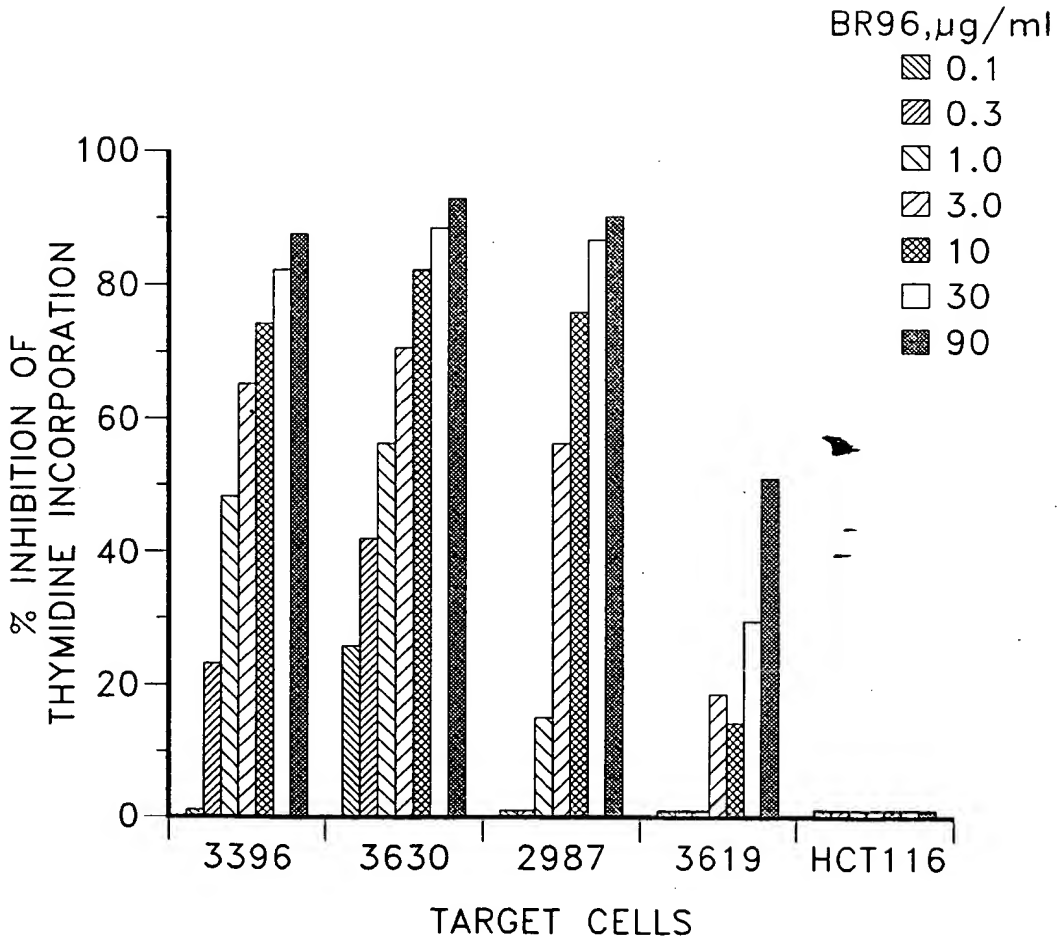

BR96,μg/ml
0.1
0.3
1.0
3.0
10
30
90
100
80
60
40
20
0
% INHIBITION OF THYMIDINE INCORPORATION
3396
3630
2987
3619
HCT116
TARGET CELLS

FIG. 7

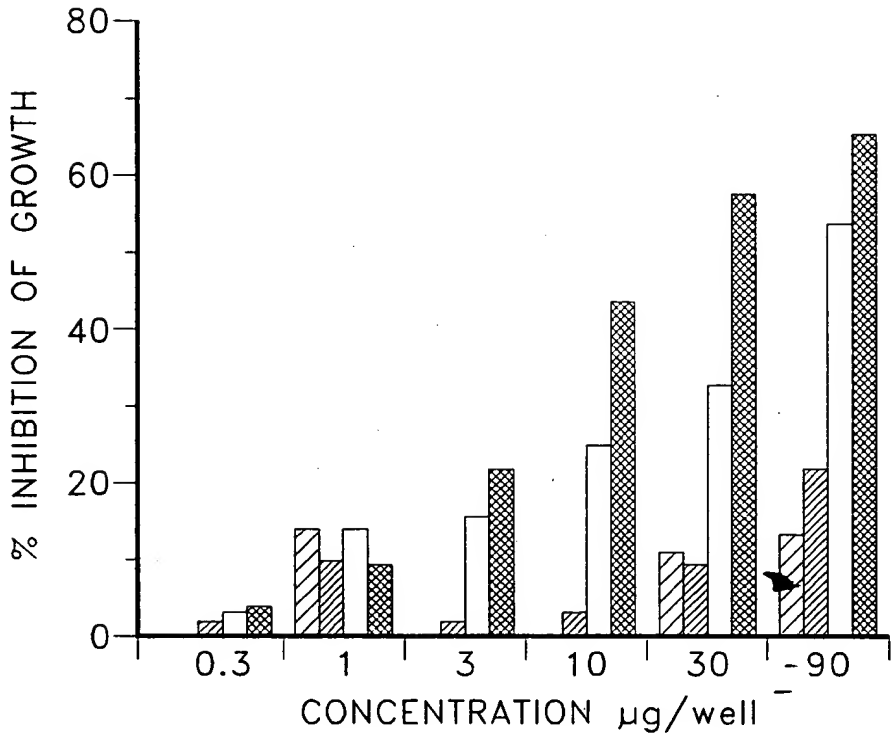

80
60
40
20
0
% INHIBITION OF GROWTH
0.3
1
3
10
30
-90
CONCENTRATION µg/well

FIG. 8

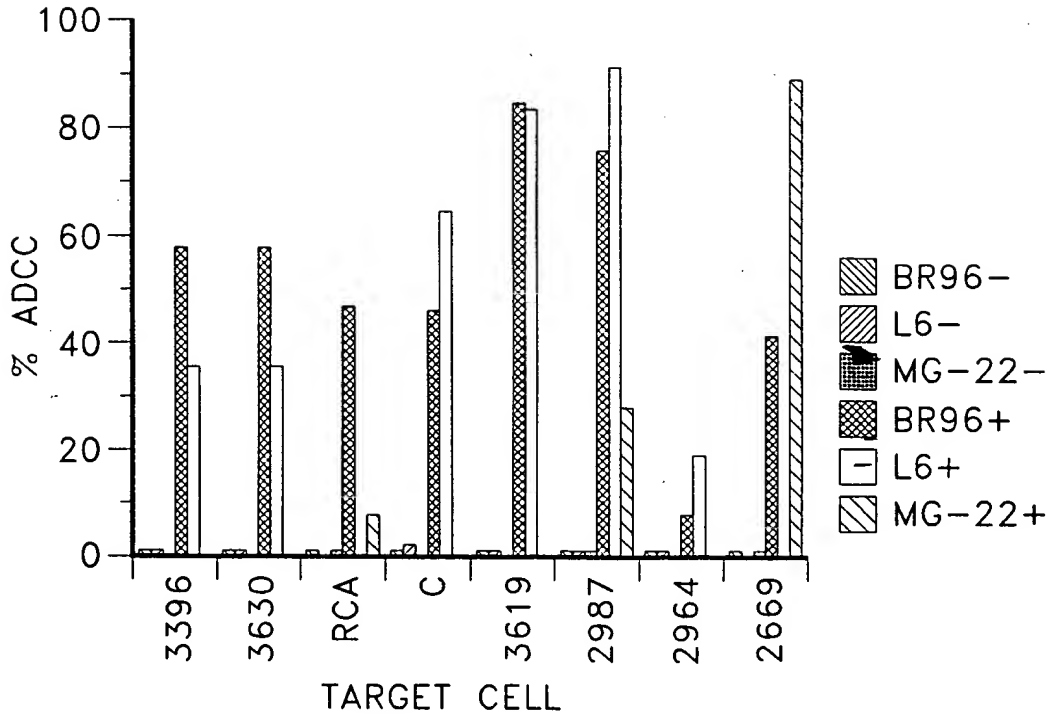

100
80
60
40
20
0
% ADCC
3396
3630
RCA
C
3619
2987
2964
2669
TARGET CELL
BR96−
L6−
MG−22−
BR96+
L6+
MG−22+

FIG. 9

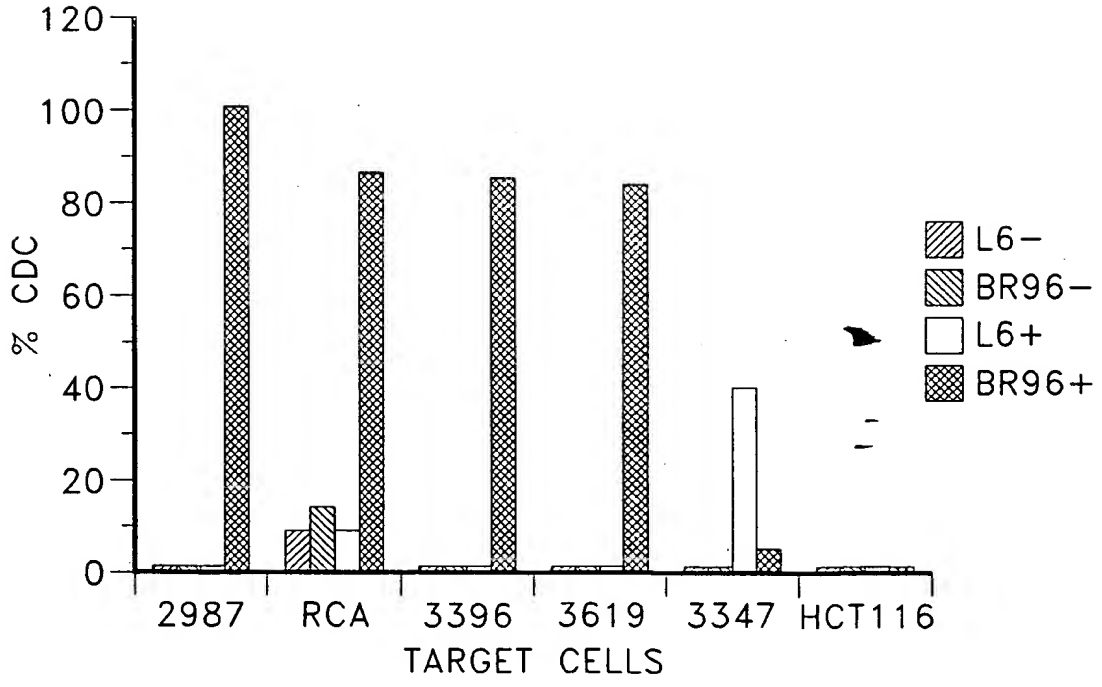

120
100
80
60
40
20
0
% CDC
2987
RCA
3396
3619
3347
HCT116
TARGET CELLS
L6−
BR96−
L6+
BR96+

FIG. 10

SUBSTITUTE SHEET

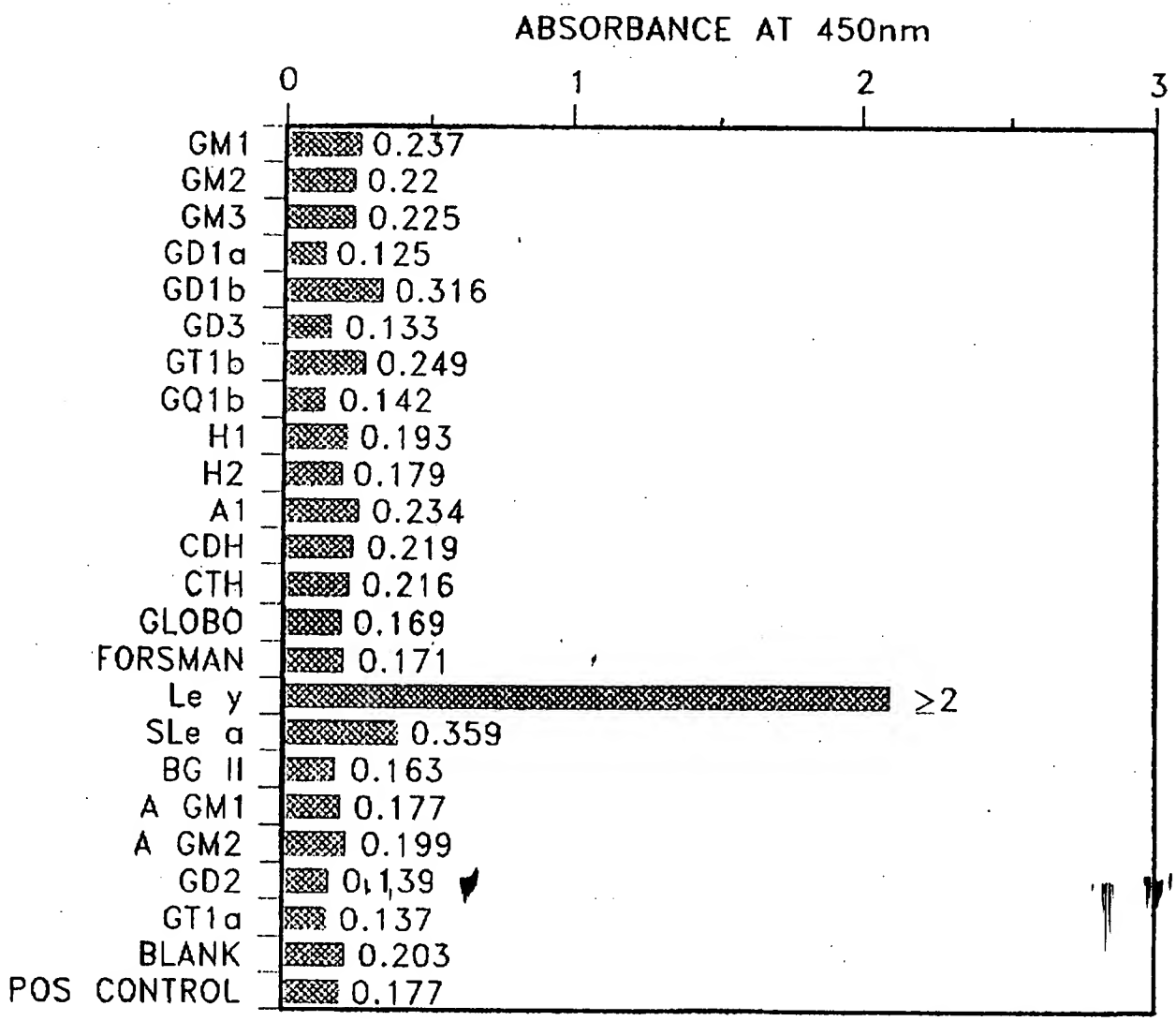


FIG. 11

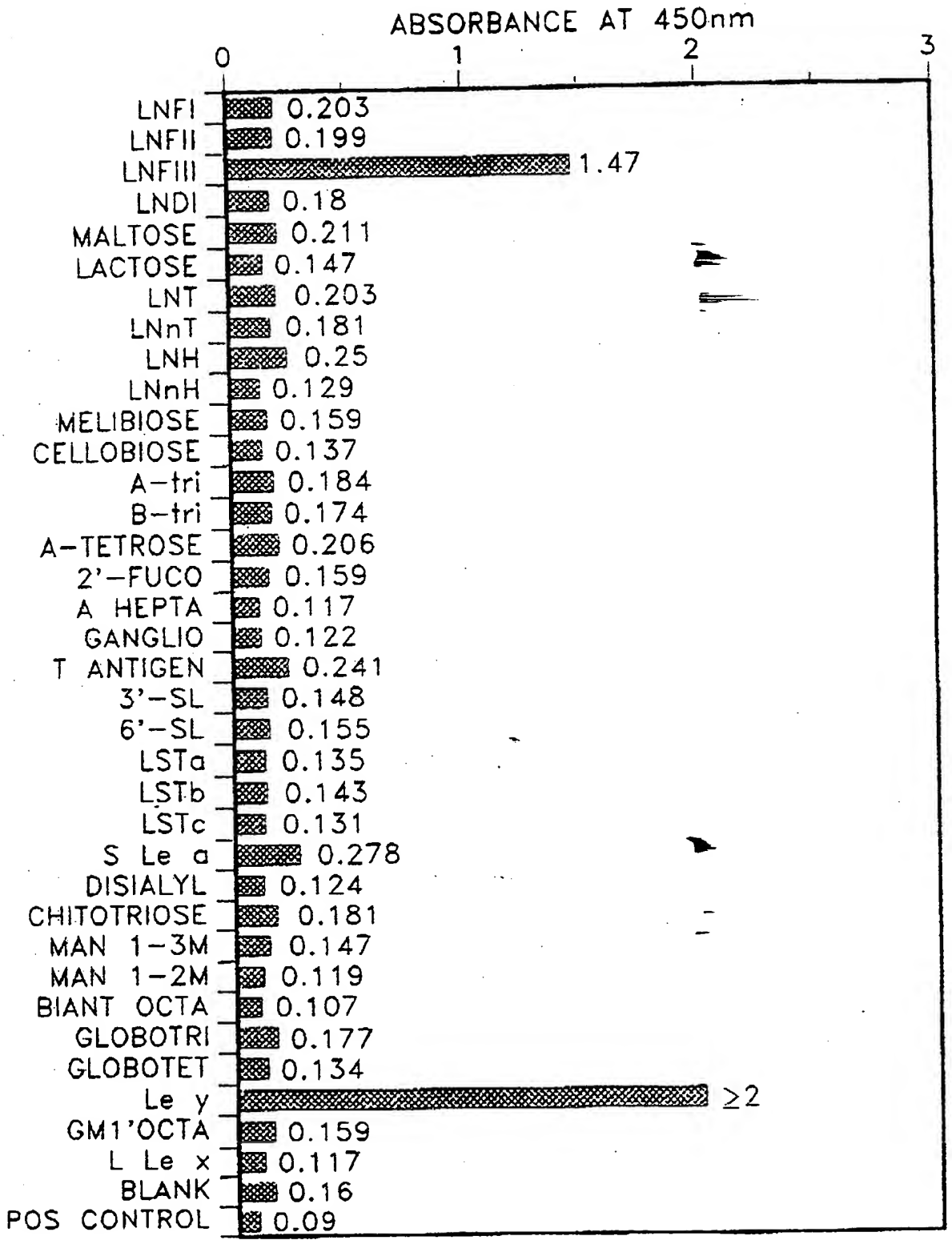

ABSORBANCE AT 450nm
0
1
2
3
LNFI 0.203
LNFII 0.199
LNFIII 1.47
LNDI 0.18
MALTOSE 0.211
LACTOSE 0.147
LNT 0.203
LNnT 0.181
LNH 0.25
LNnH 0.129
MELIBIOSE 0.159
CELLOBIOSE 0.137
A-tri 0.184
B-tri 0.174
A-TETROSE 0.206
2'-FUCO 0.159
A HEPTA 0.117
GANGLIO 0.122
T ANTIGEN 0.241
3'-SL 0.148
6'-SL 0.155
LSTa 0.135
LSTb 0.143
LSTc 0.131
S Le a 0.278
DISIALYL 0.124
CHITOTRIOSE 0.181
MAN 1-3M 0.147
MAN 1-2M 0.119
BIANT OCTA 0.107
GLOBOTRI 0.177
GLOBOTET 0.134
Le y ≥2
GM1'OCTA 0.159
L Le x 0.117
BLANK 0.16
POS CONTROL 0.09

FIG. 12

66ETh0" 96Z06Z60

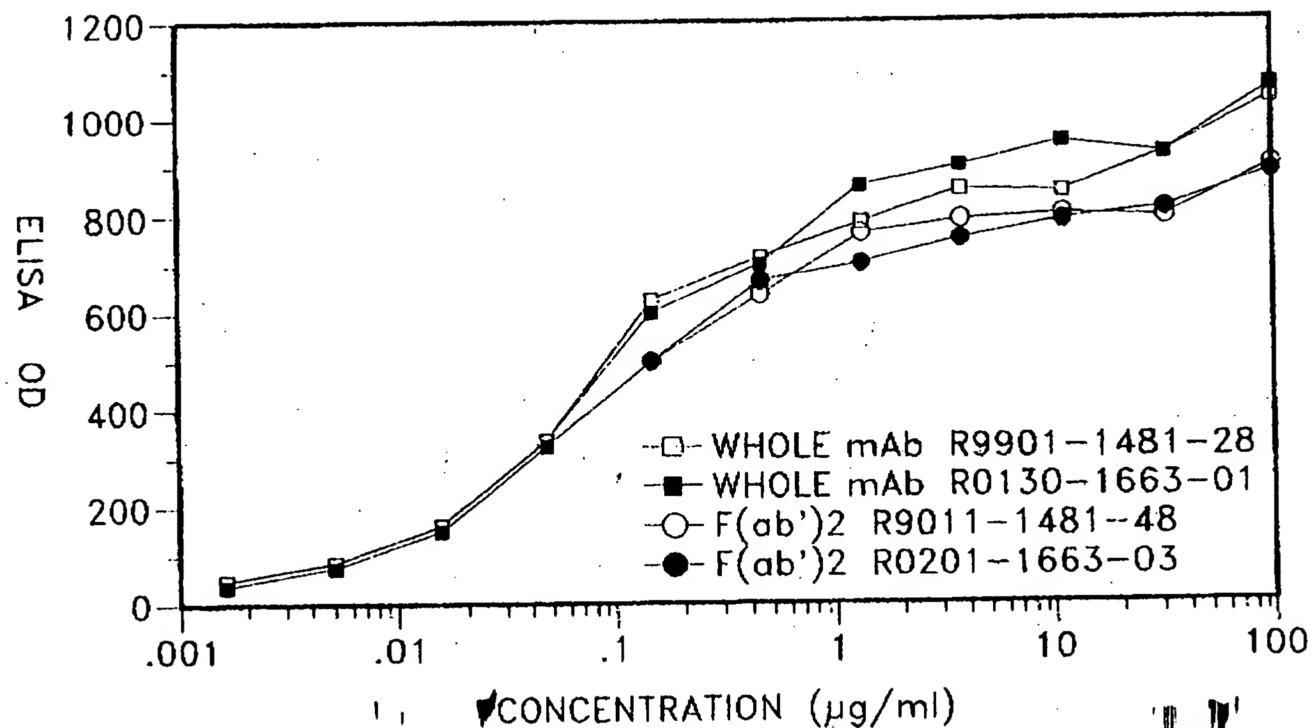


FIG. 13

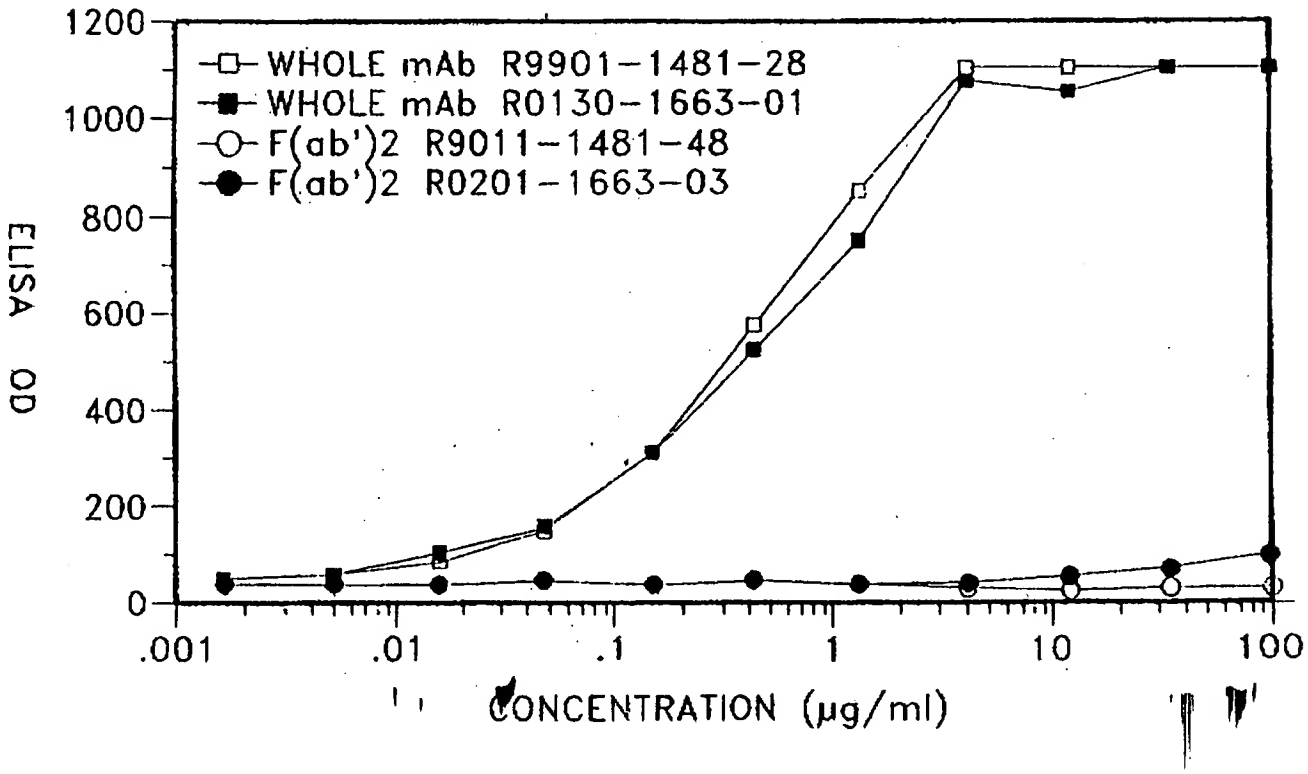

WHOLE mAb R9901-1481-28
WHOLE mAb R0130-1663-01
F(ab')2 R9011-1481-48
F(ab')2 R0201-1663-03
ELISA OD
1200
1000
800
600
400
200
0
.001
.01
.1
1
10
100
CONCENTRATION (µg/ml)

FIG. 14

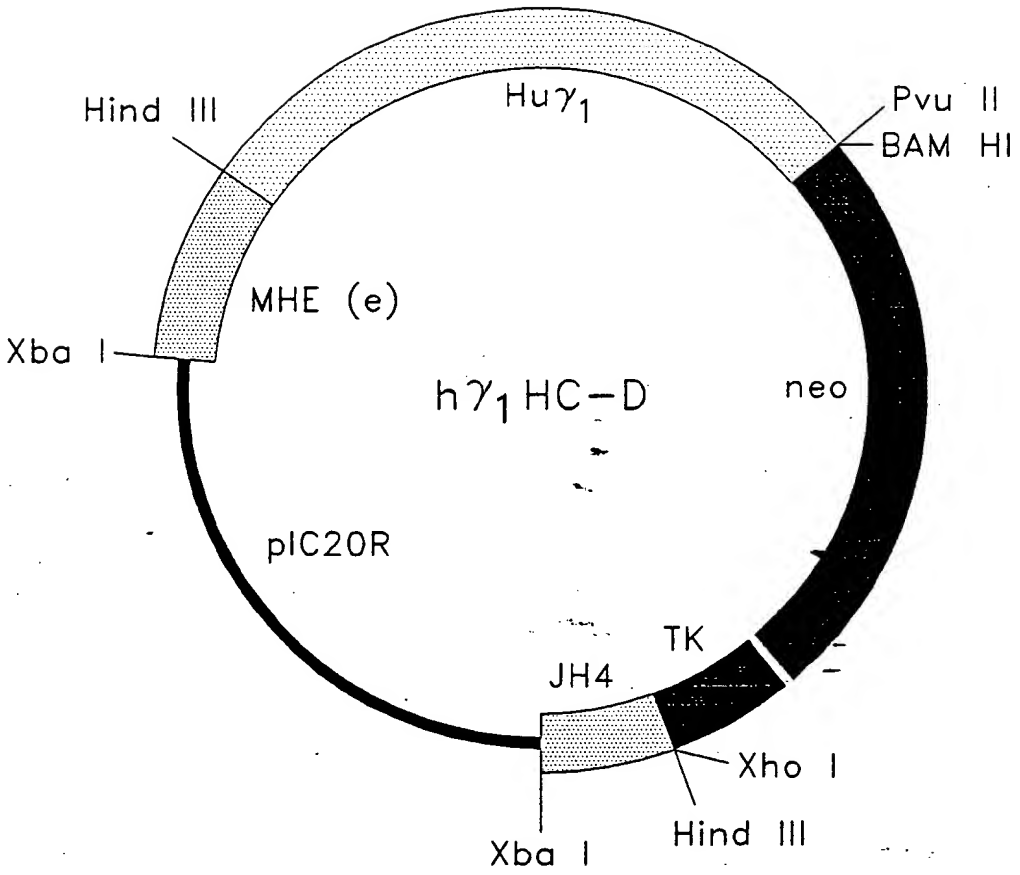

Hind III
Huγ1
Pvu II
BAM HI
MHE (e)
Xba I
hγ1HC-D
neo
pIC20R
TK
JH4
Xho I
Hind III
Xba I

FIG. 15

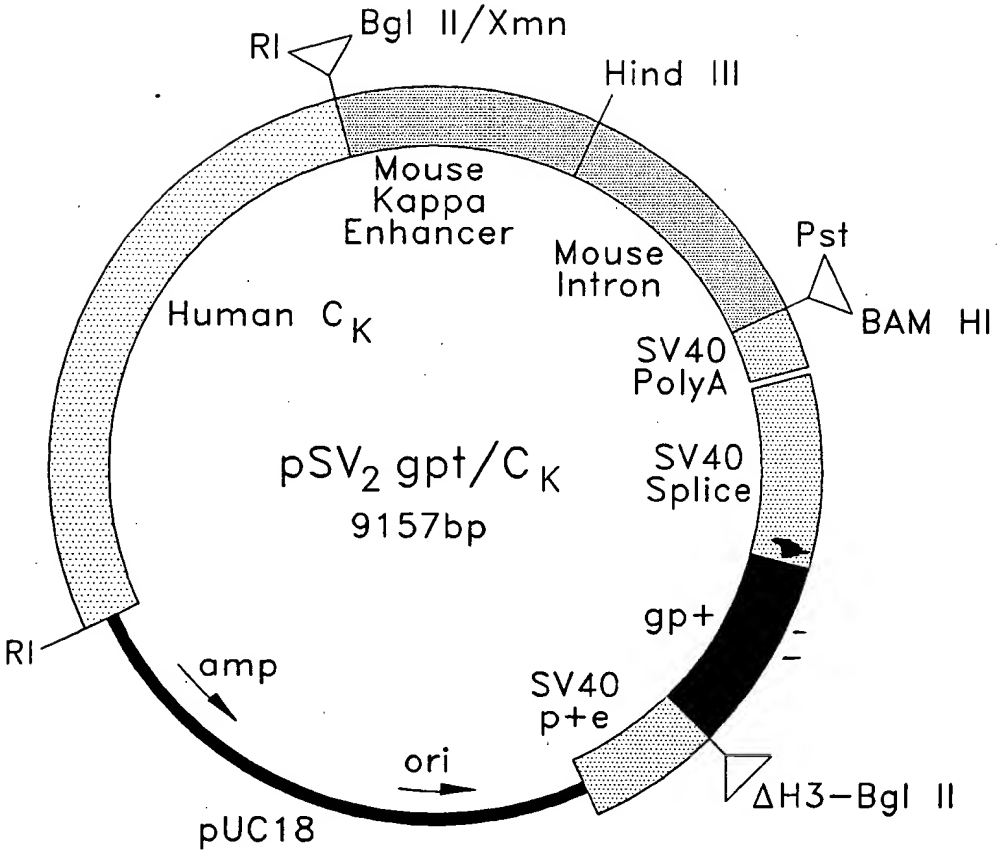

RI
Bgl II/Xmn
Hind III
Mouse
Kappa
Enhancer
Mouse
Intron
Pst
BAM HI
Human C_K
SV40
PolyA
pSV_2 gpt/C_K
9157bp
SV40
Splice
gp+
RI
amp
SV40
p+e
ori
ΔH3–Bgl II
pUC18

FIG. 16

CHARACTERIZATION OF CHIMERIC ANTIBODY SPECIFICITY AND AFFINITY BY A COMPETITION BINDING ASSAY

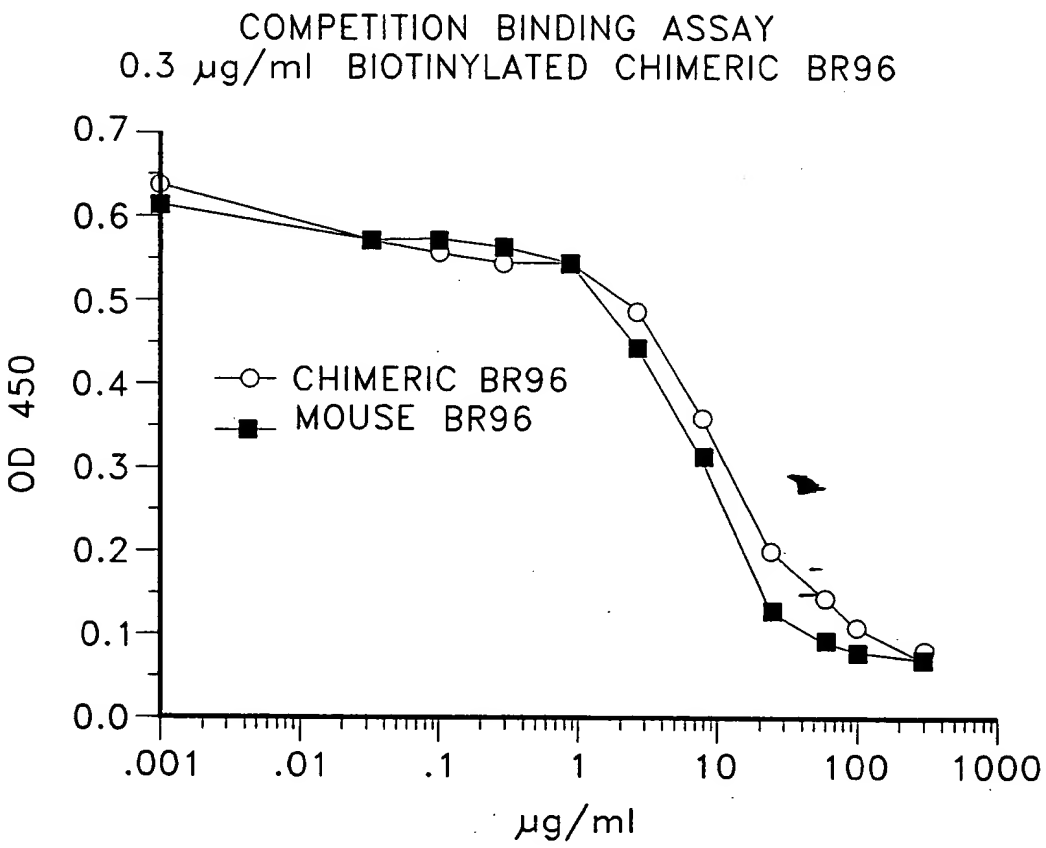


FIG. 17

86ETHO 86206360

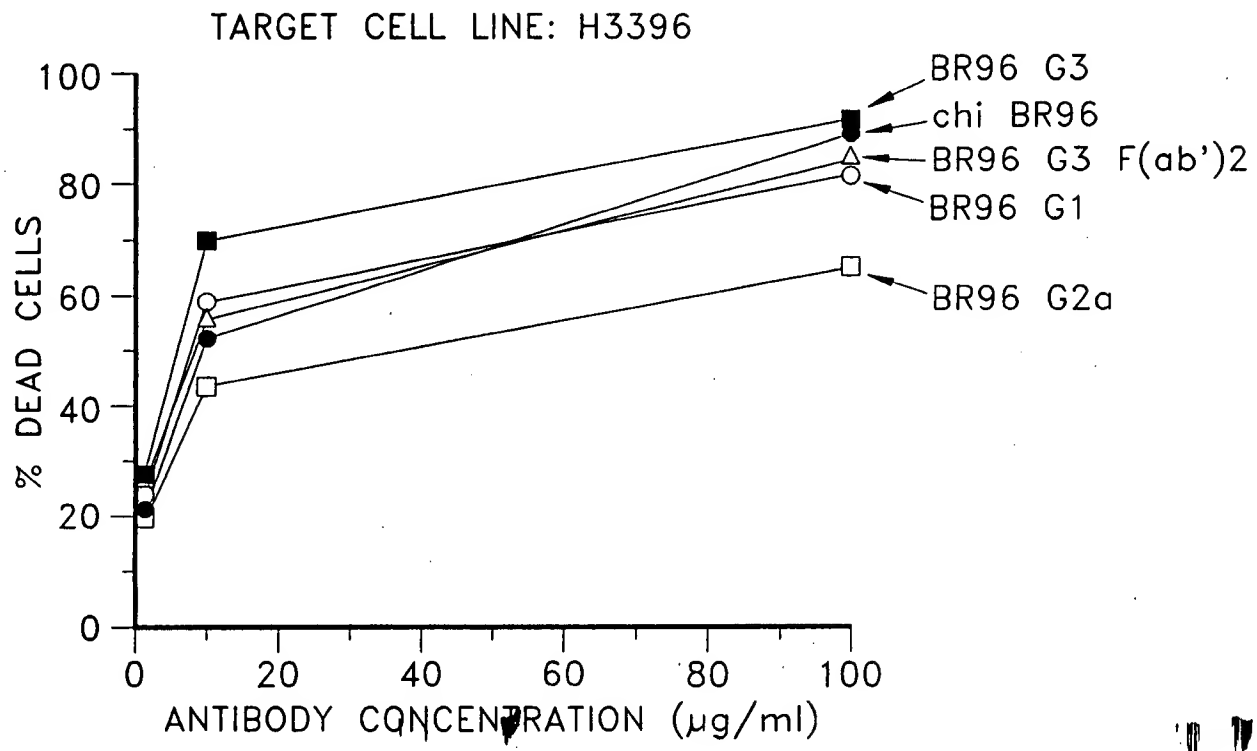


FIG. 18

66ETHD" 86206260

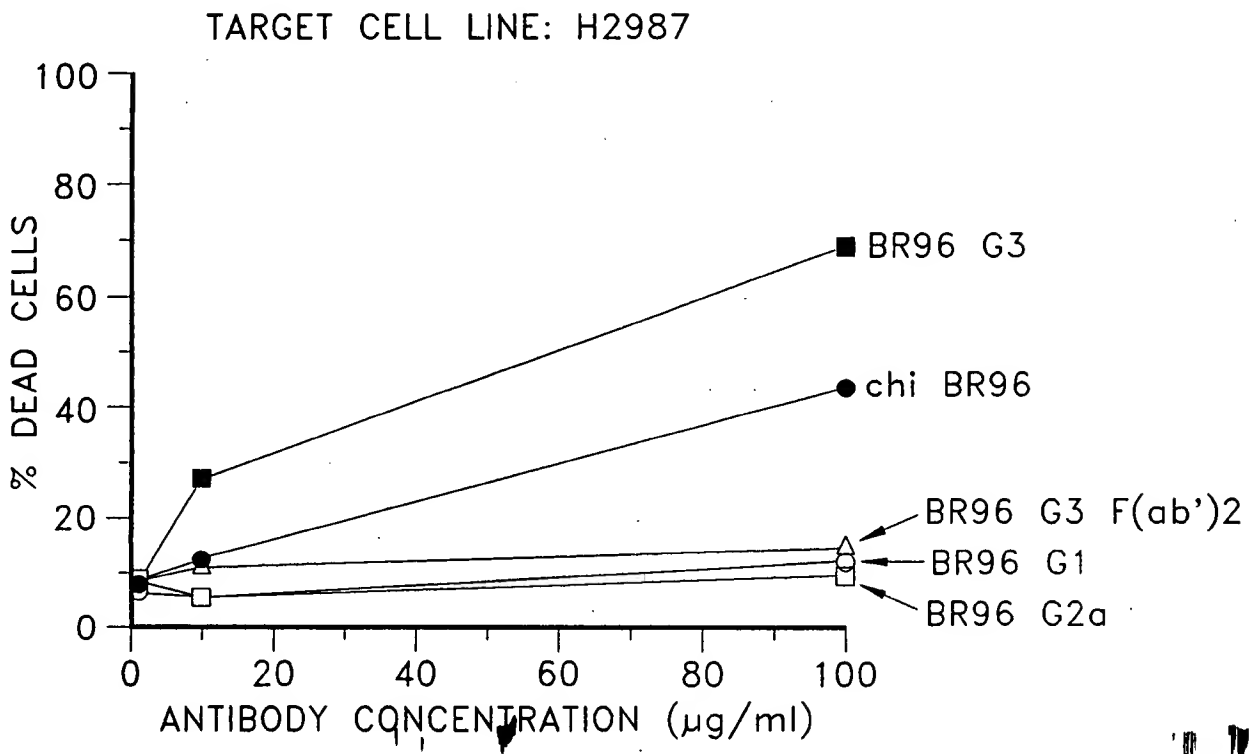


FIG. 19

SUBSTITUTE SHEET

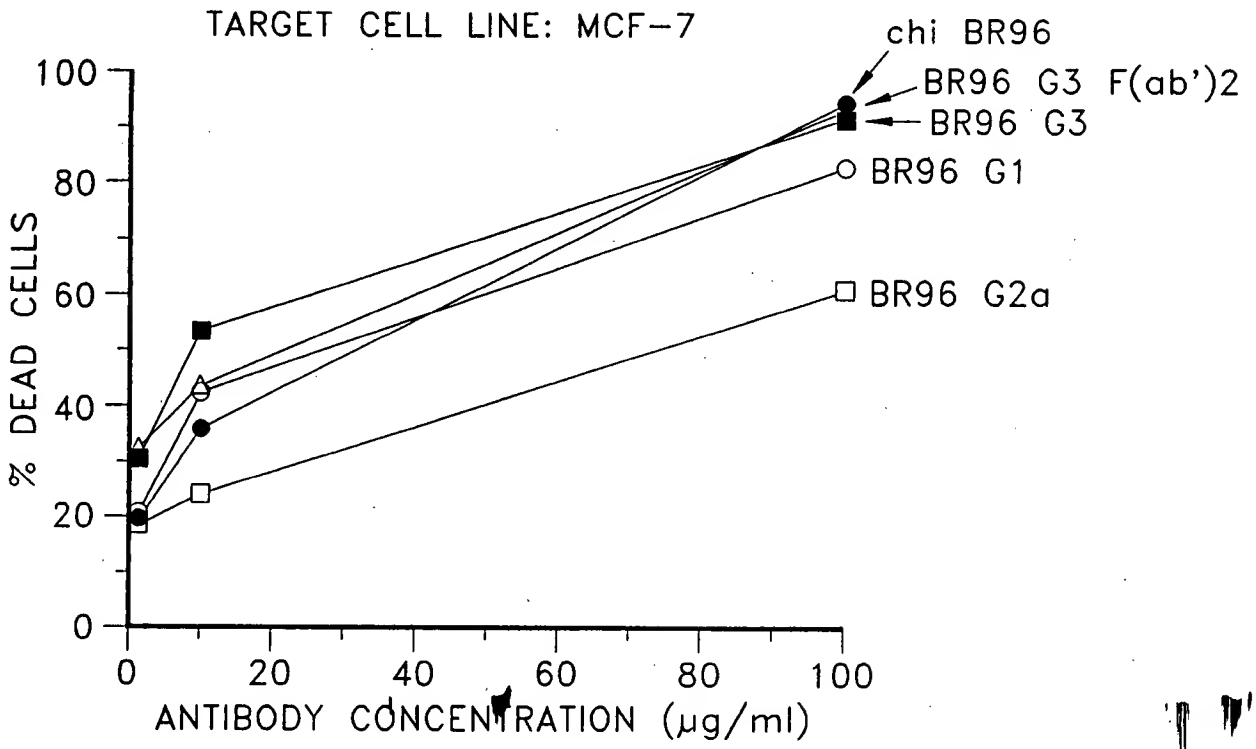


FIG. 20

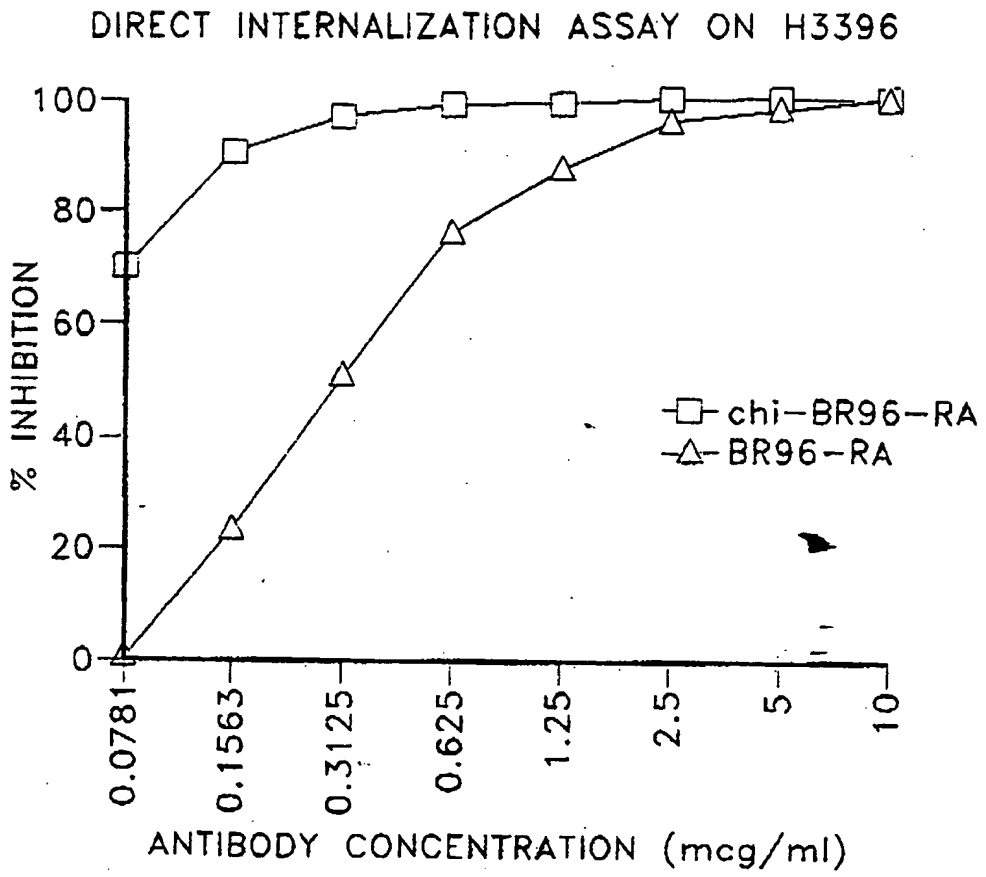

DIRECT INTERNALIZATION ASSAY ON H3396
% INHIBITION
100
80
60
40
20
0
0.0781
0.1563
0.3125
0.625
1.25
2.5
5
10
ANTIBODY CONCENTRATION (mcg/ml)
chi-BR96-RA
BR96-RA

FIG. -21

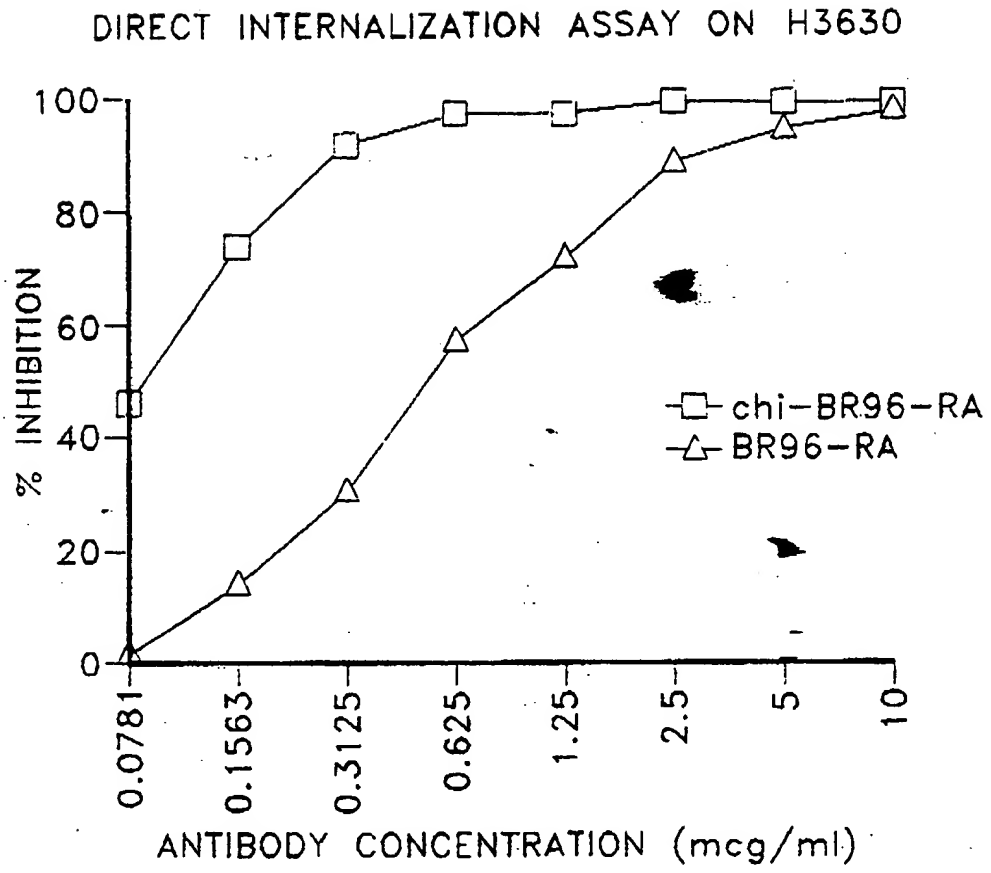

DIRECT INTERNALIZATION ASSAY ON H3630
% INHIBITION
100
80
60
40
20
0
0.0781
0.1563
0.3125
0.625
1.25
2.5
5
10
ANTIBODY CONCENTRATION (mcg/ml)
chi-BR96-RA
BR96-RA

FIG. 22

6667470 "26206260

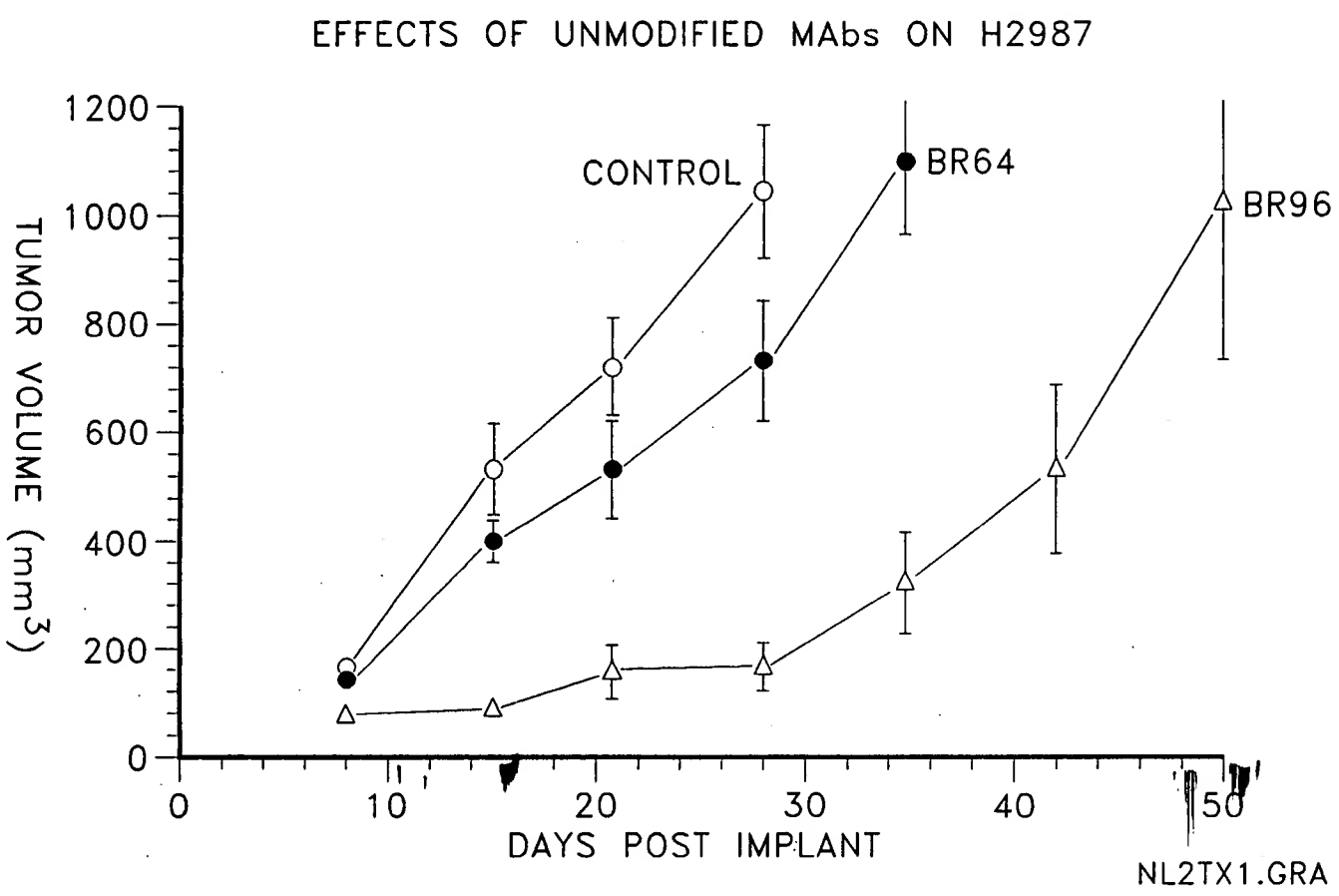


FIG. 23

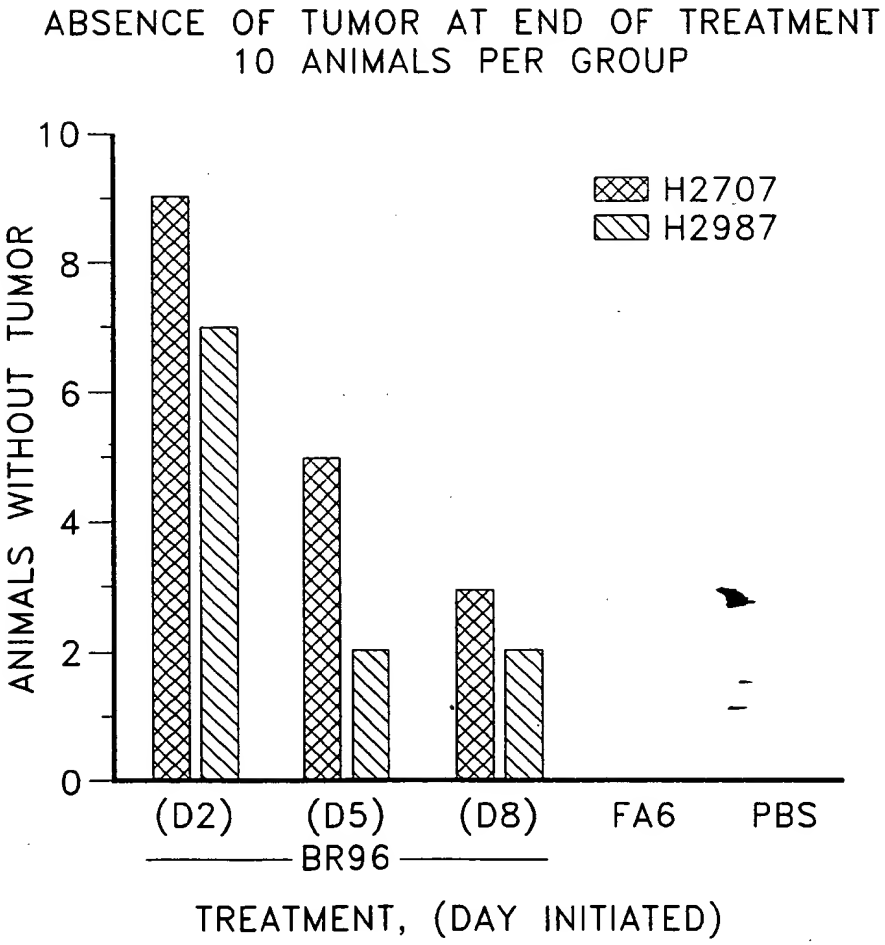

ABSENCE OF TUMOR AT END OF TREATMENT
10 ANIMALS PER GROUP
ANIMALS WITHOUT TUMOR
10
8
6
4
2
0
H2707
H2987
(D2)
(D5)
(D8)
FA6
PBS
BR96
TREATMENT, (DAY INITIATED)

FIG. 24

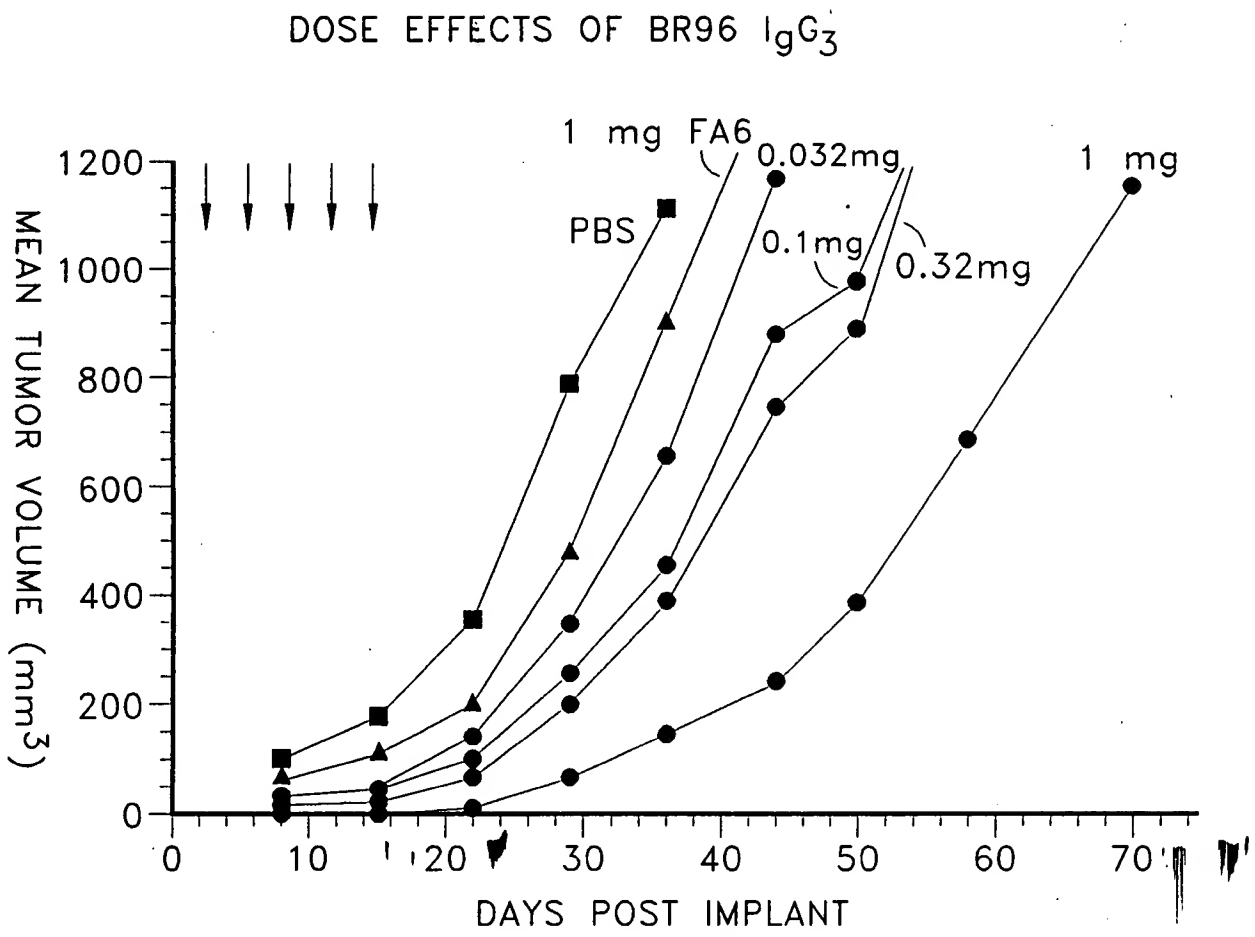

DOSE EFFECTS OF BR96 IgG3
1 mg FA6
0.032mg
1 mg
PBS
0.1mg
0.32mg
MEAN TUMOR VOLUME (mm3)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
70
DAYS POST IMPLANT

FIG. 25

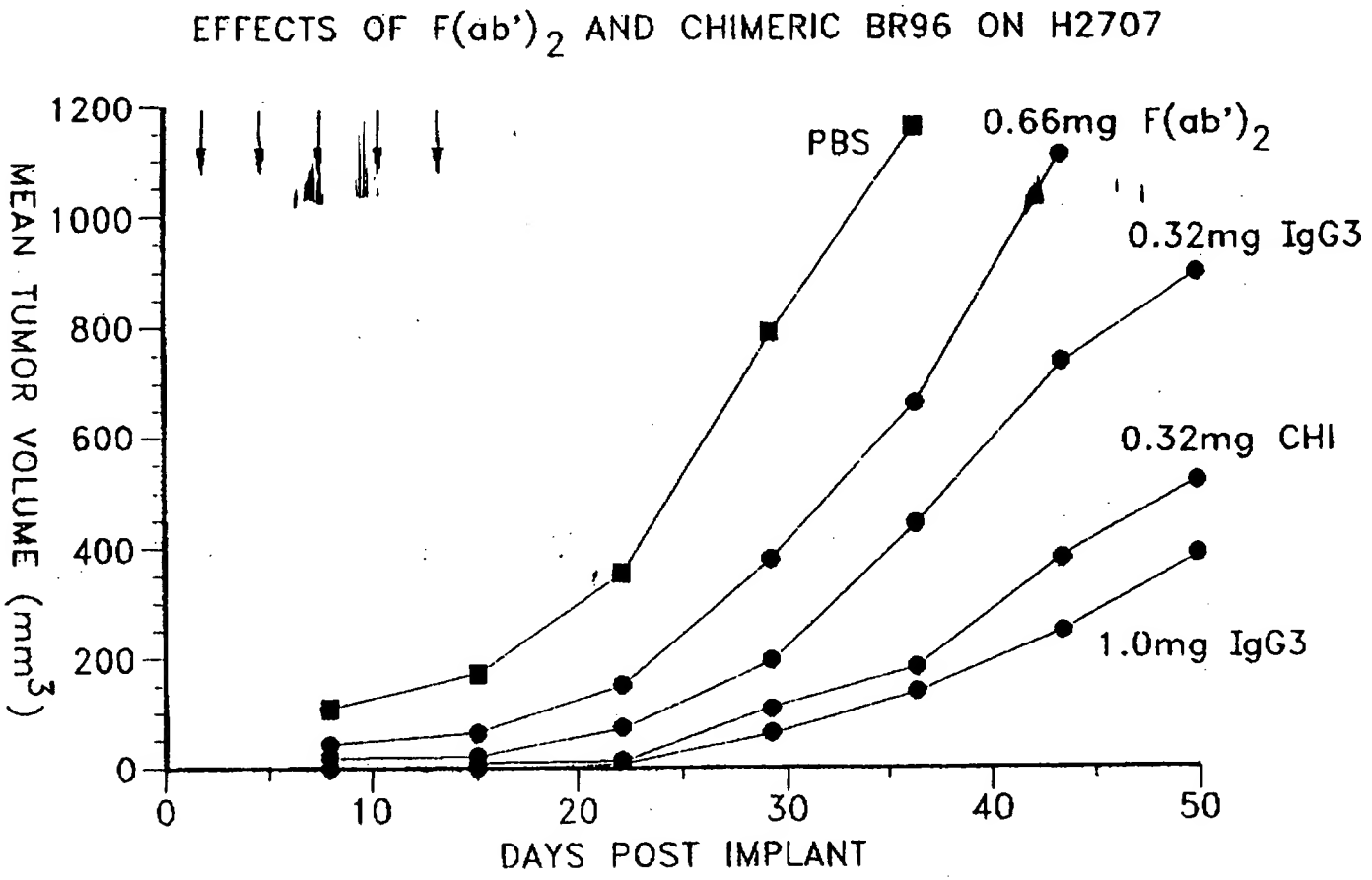

EFFECTS OF F(ab')2 AND CHIMERIC BR96 ON H2707
MEAN TUMOR VOLUME (mm3)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
DAYS POST IMPLANT
PBS
0.66mg F(ab')2
0.32mg IgG3
0.32mg CHI
1.0mg IgG3

FIG. 26

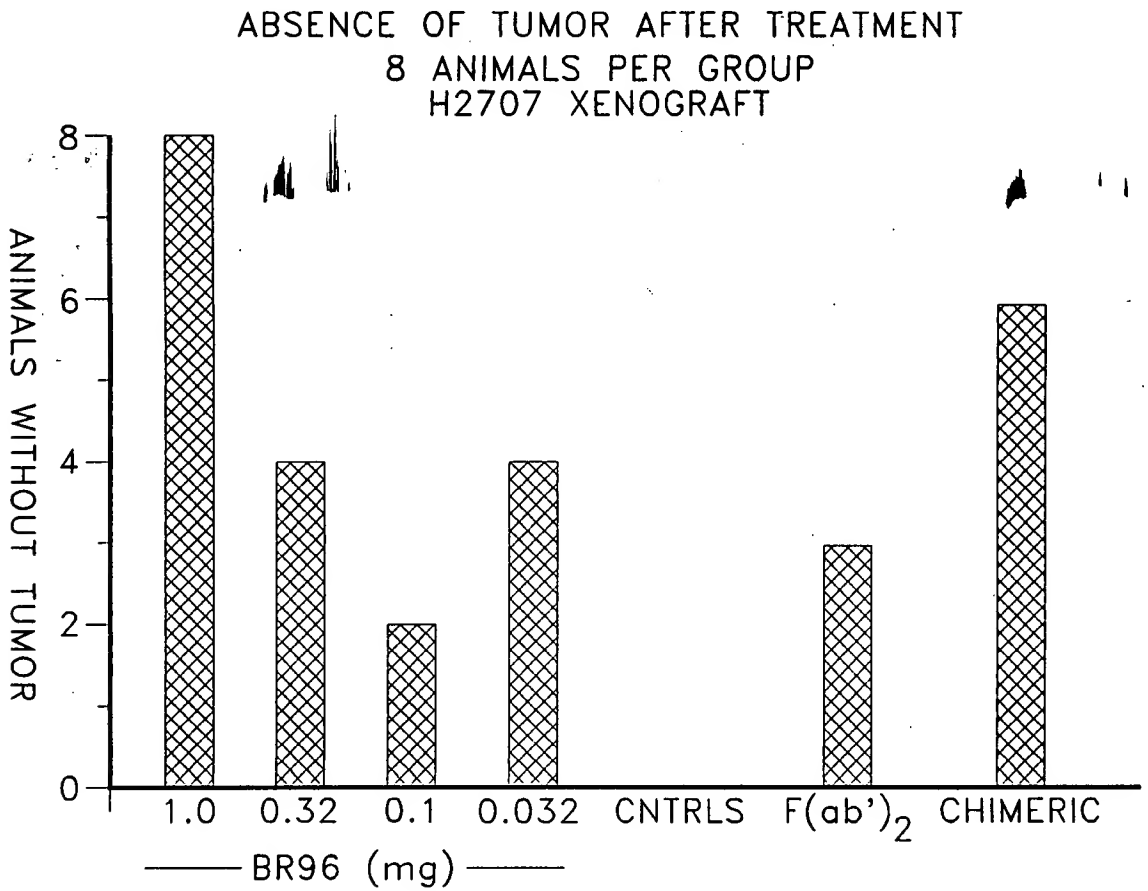


FIG. 27

0292786 B1

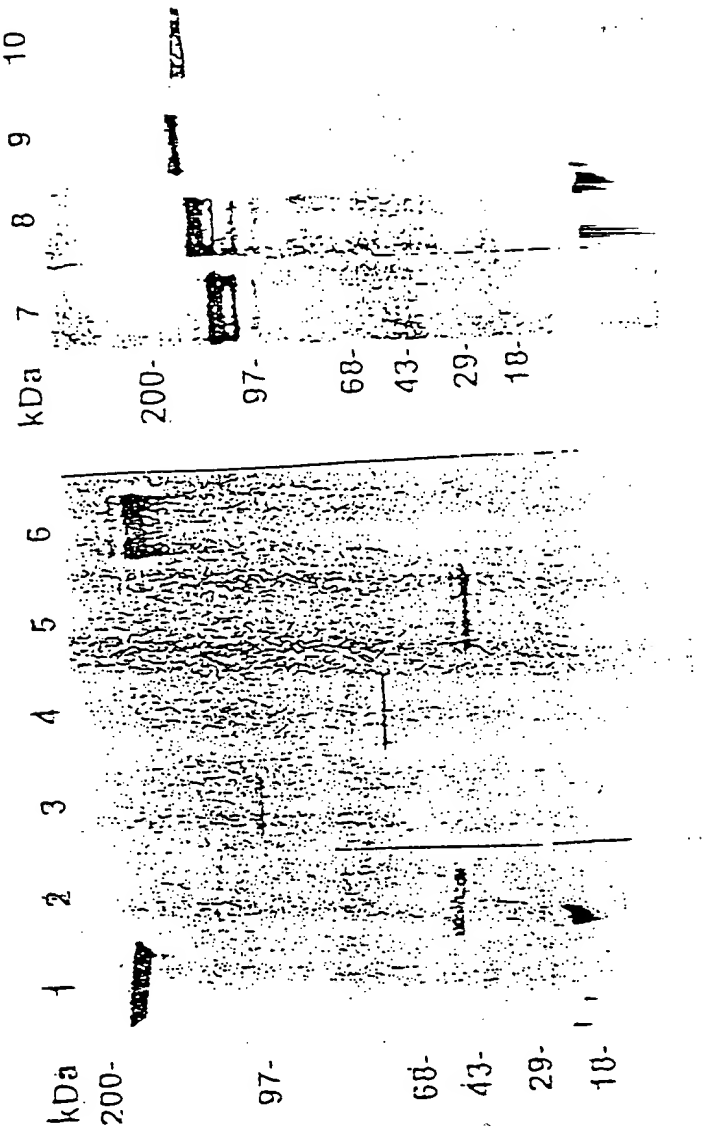


FIGURE 28

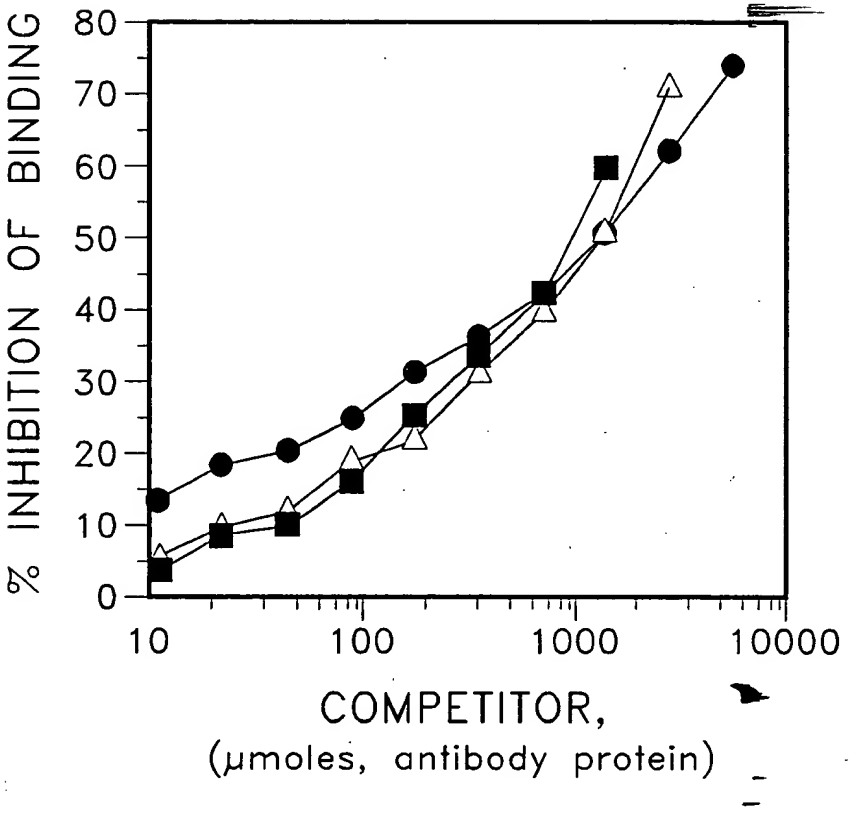

% INHIBITION OF BINDING
0
10
20
30
40
50
60
70
80
10
100
1000
10000
COMPETITOR,
(μmoles, antibody protein)

FIG. 29

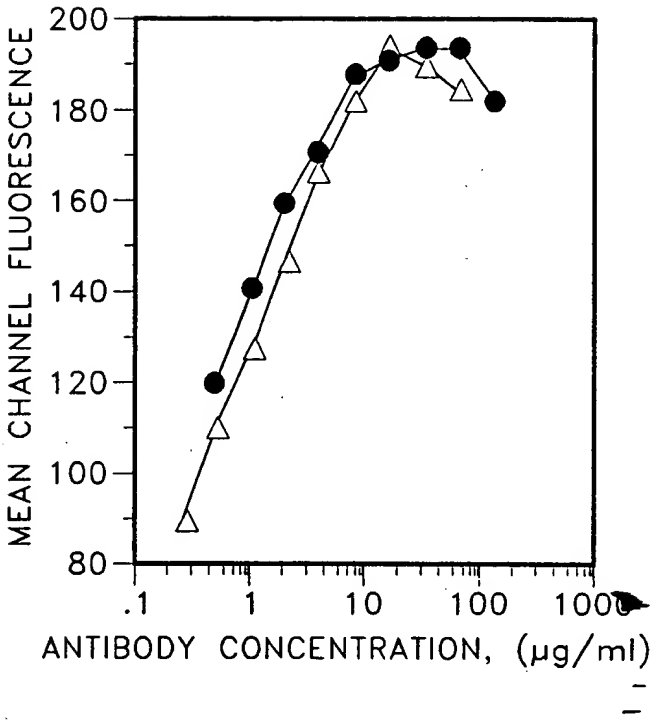

MEAN CHANNEL FLUORESCENCE
200
180
160
140
120
100
80
.1
1
10
100
1000
ANTIBODY CONCENTRATION, (μg/ml)

FIG. 30A

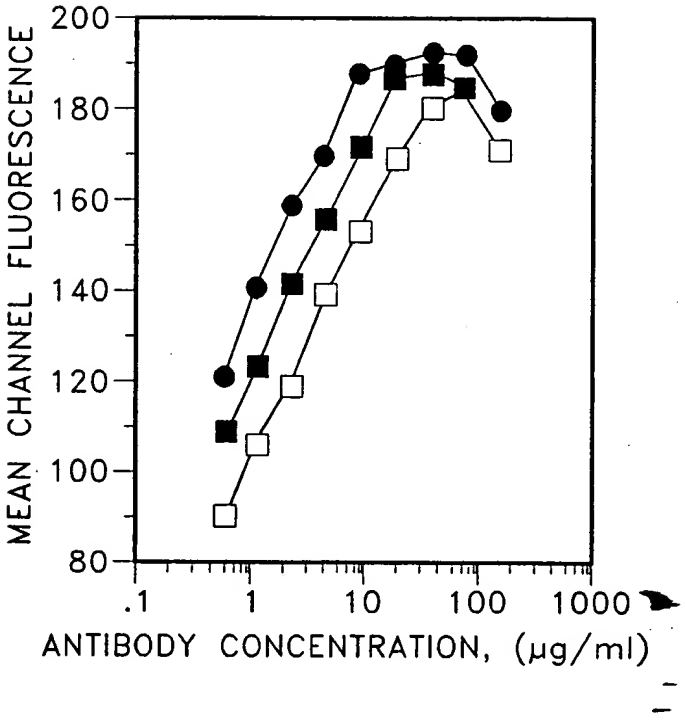

MEAN CHANNEL FLUORESCENCE
200
180
160
140
120
100
80
.1
1
10
100
1000
ANTIBODY CONCENTRATION, (μg/ml)

FIG. 30B

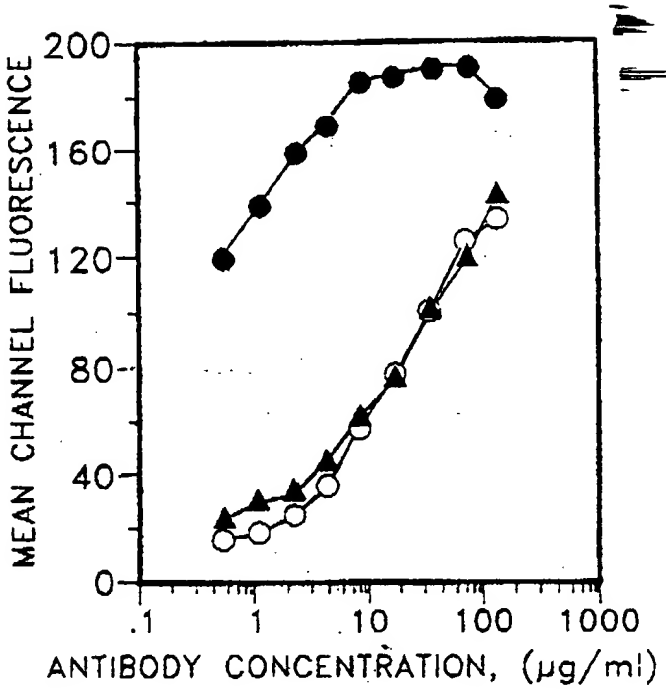

MEAN CHANNEL FLUORESCENCE
200
160
120
80
40
0
.1
1
10
100
1000
ANTIBODY CONCENTRATION, (μg/ml)

FIG. 30C

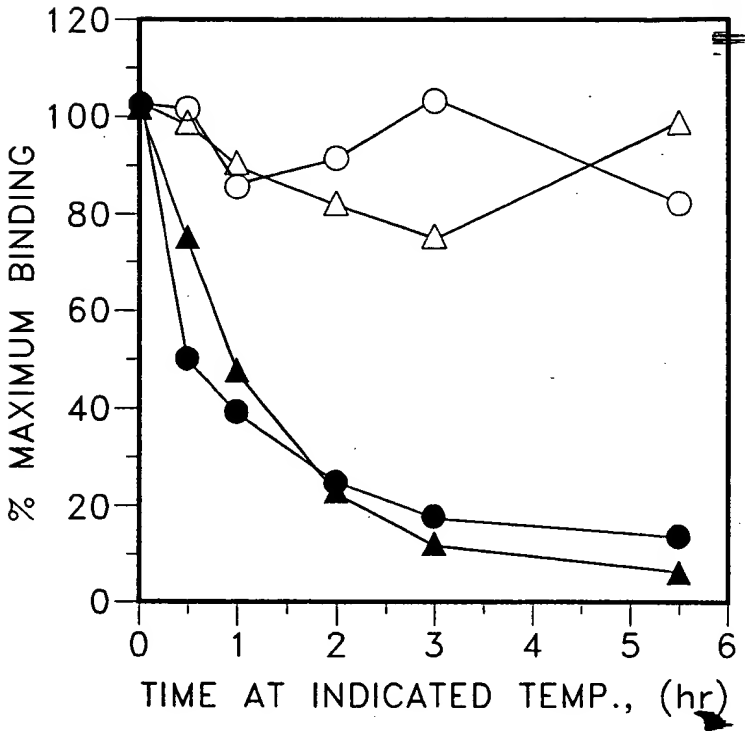

120
100
80
60
40
20
0
% MAXIMUM BINDING
0
1
2
3
4
5
6
TIME AT INDICATED TEMP., (hr)

FIG. 31A

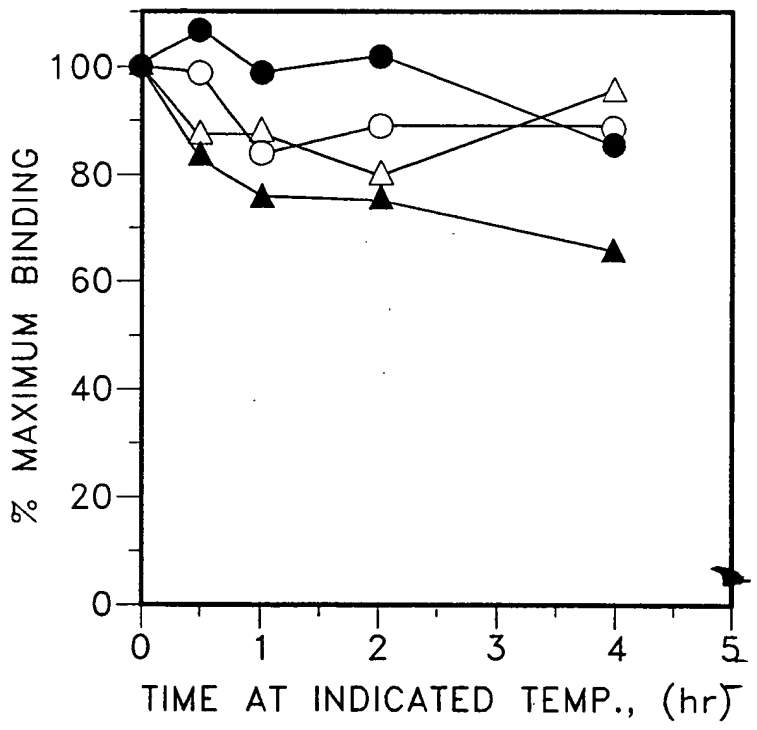

100
80
60
40
20
0
% MAXIMUM BINDING
0
1
2
3
4
5
TIME AT INDICATED TEMP., (hr)

FIG. 31B

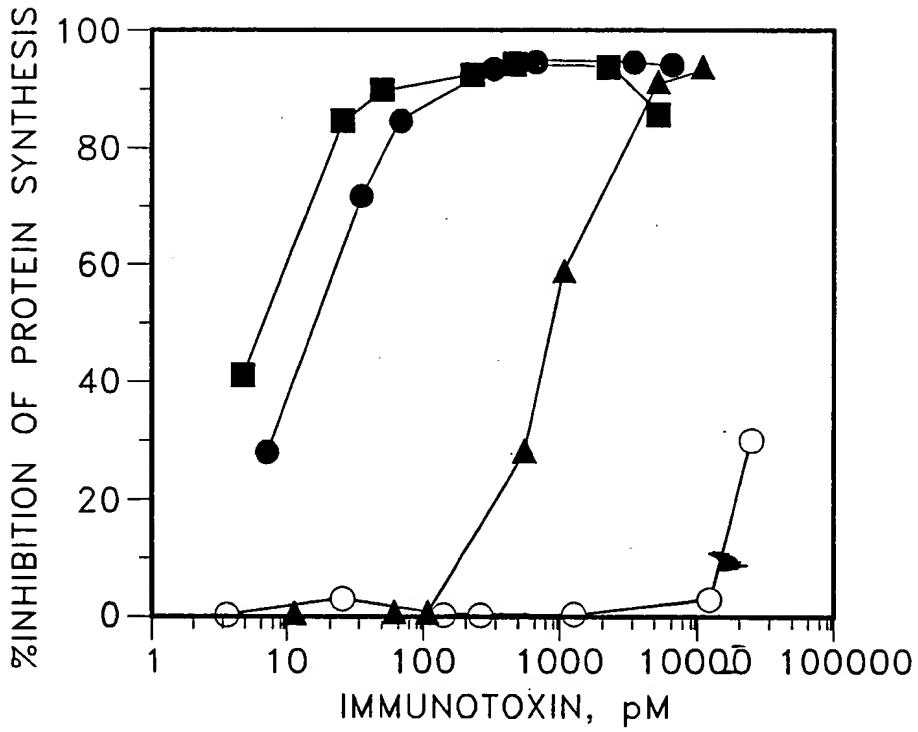

%INHIBITION OF PROTEIN SYNTHESIS
100
80
60
40
20
0
1
10
100
1000
10000
100000
IMMUNOTOXIN, pM

FIG. 32

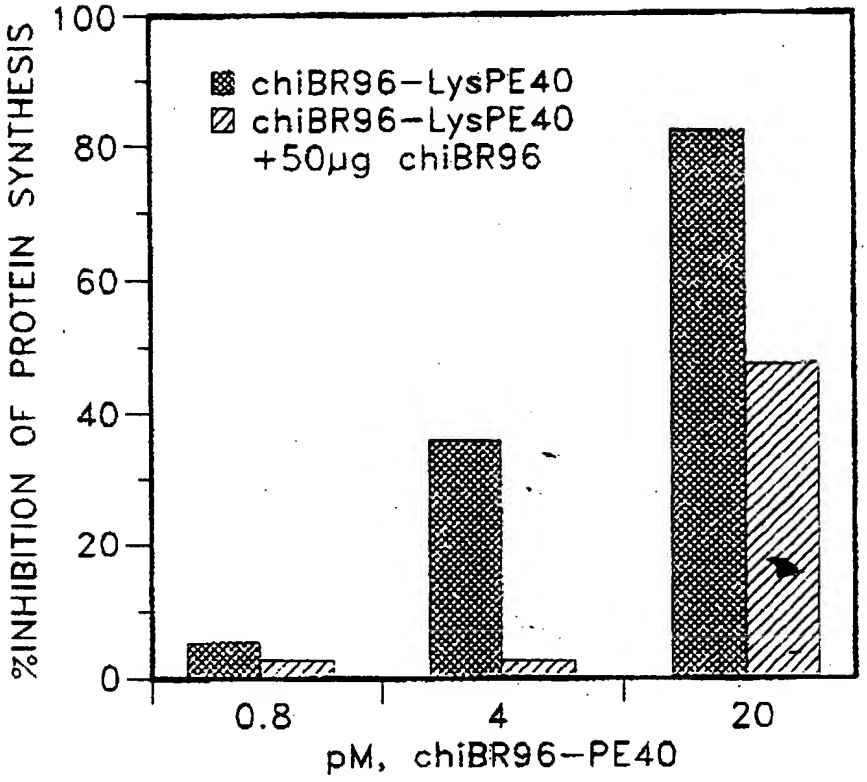

%INHIBITION OF PROTEIN SYNTHESIS
100
80
60
40
20
0
chiBR96–LysPE40
chiBR96–LysPE40
+50µg chiBR96
0.8
4
20
pM, chiBR96–PE40

FIG. 33

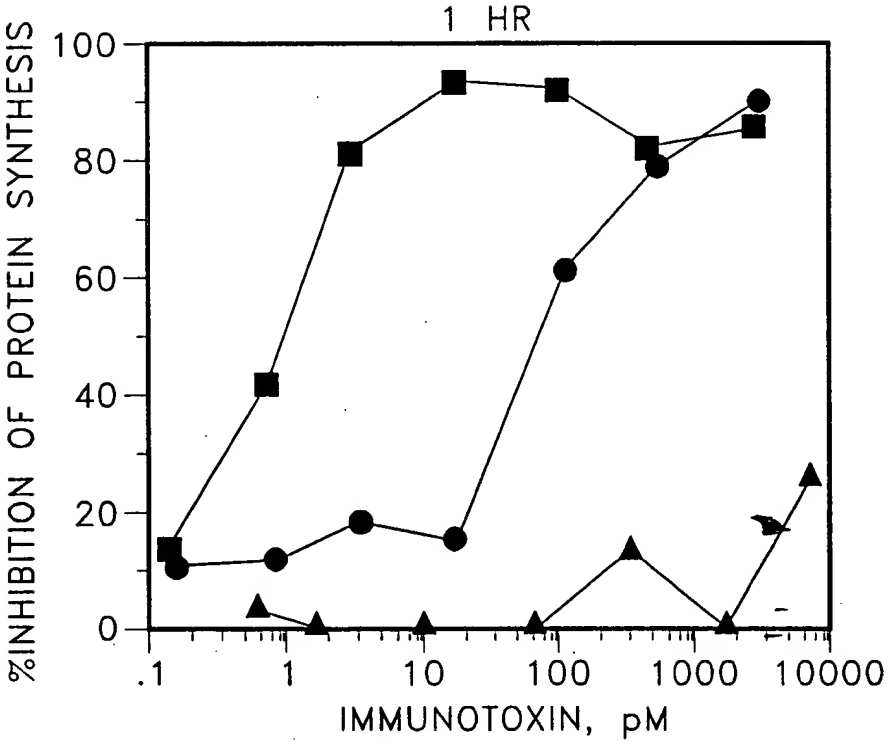

1 HR
%INHIBITION OF PROTEIN SYNTHESIS
100
80
60
40
20
0
.1
1
10
100
1000
10000
IMMUNOTOXIN, pM

FIG. 34A

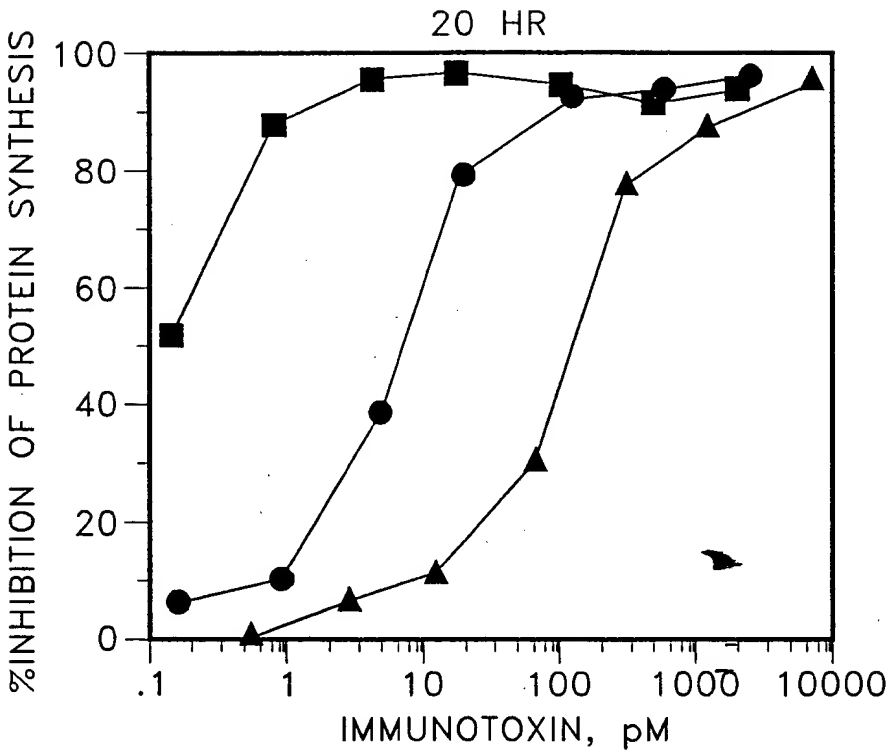

20 HR
%INHIBITION OF PROTEIN SYNTHESIS
100
80
60
40
20
0
.1
1
10
100
1000
10000
IMMUNOTOXIN, pM

FIG. 34B

```
            10          20          30          40
ATG GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTA GTG CAG CCT GGG
Met Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

50          60          70          80          90
TCC CTG AAA GTC TCC TGT GTA ACC TCT GGA TTC ACT TTC AGT GAC TAT
Ser Leu Lys Val Ser Cys Val Thr Ser Gly Phe Thr Phe Ser Asp Tyr

  100         110         120         130         140
TAC ATG TGG GTT CGC CAG ACT CCA GAG AAG AGG CTG GAG TGG GTC GCA
Tyr Met Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala

     150         160         170         180         190
TAC ATT AGT CAA GGT GAT ATA ACC GAC TAT CCA GAC ACT GTA AAG GGT
Tyr Ile Ser Gln Gly Asp Ile Thr Asp Tyr Pro Asp Thr Val Lys Gly

        200         210         220         230         240
CGA TTC ACC ATC TCC AGA GAC AAT AAG AAC ACC CTG TAC CTG CAA ATG
Arg Phe Thr Ile Ser Arg Asp Asn Lys Asn Thr Leu Tyr Leu Gln Met

          250         260         270         280
AGC CGT CTG AAG TCT GAG GAC ACA GCC ATG TAT TGT GCA AGA GGC CTG
Ser Arg Leu Lys Ser Glu Asp Thr Ala Met Tyr Cys Ala Arg Gly Leu

290         300         310         320         330
GAC GAC GGG GCC TGG TTT GCT TAC TGG GGC CAA GGG ACC ACG ACC GTC
Asp Asp Gly Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr Thr Thr Val

  340         350         360         370         380
TCC TCA GGA TCC GGA GGT GGA GGT TCT GGT GGA GGT GGA TCT GGA GGT
Ser Ser Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly

     390         400         410         420         430
GGA TCT AAG CTT GAT GTT TTG ATG ACC CAA ATT CCA GTC TCC CTG CCT
Gly Ser Lys Leu Asp Val Leu Met Thr Gln Ile Pro Val Ser Leu Pro

        440         450         460         470         480
GTC AGT CTT GGA CAA GCG TCC ATC TCT TGC AGA TCT AGT CAG ATC ATT
Val Ser Leu Gly Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile
```

```
          490           500           510           520

GTA CAT AAT AAT GGC AAC ACC TTA GAA TGG TAC CTG CAG AAA CCA GGC
Val His Asn Asn Gly Asn Thr Leu Glu Trp Tyr Leu Gln Lys Pro Gly

530           540           550           560           570

CAG TCT CCA CAG CTC CTG ATC TAC AAA GTT AAC CGA TTT TCT GGG GTC
Gln Ser Pro Gln Leu Leu Ile Tyr Lys Val Asn Arg Phe Ser Gly Val

   580           590           600           610           620

CCA GAC AGG TTC AGC GGC AGT GGA TCA GGG ACA GAT TTC CTC AAG ATC
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Leu Lys Ile

     630           640           650           660           670

AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC TTT CAA GTT
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Val

         680           690           700           710           720

CAT GTT CCA TTC ACG TTC GGC TCG GGG ACC AAG CTG GAG ATC AAA CGC
His Val Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
```

Figure 35 (Cont.)

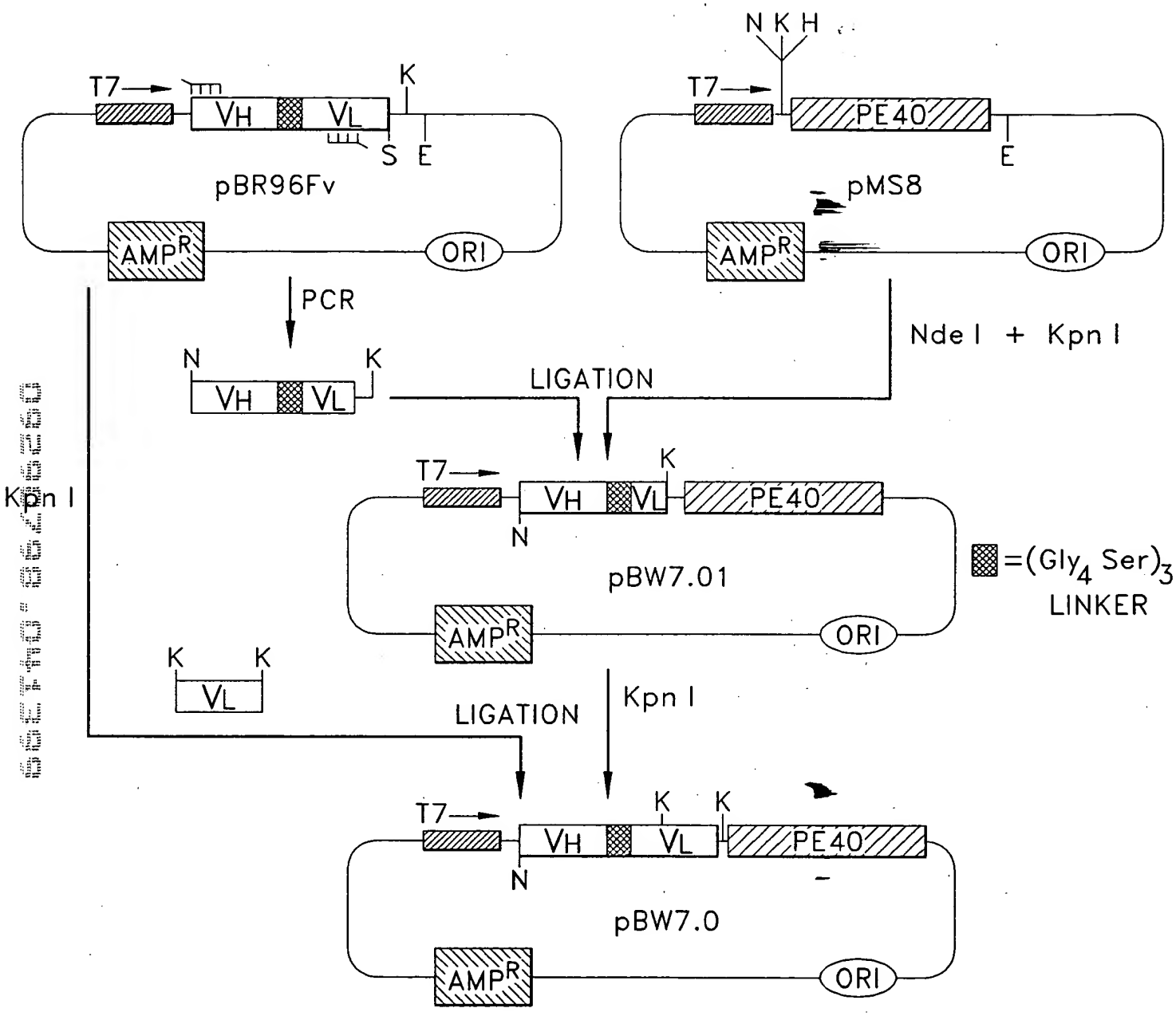

T7
VH
VL
K
S
E
pBR96Fv
AMPR
ORI
PCR
N
K
LIGATION
N K H
PE40
pMS8
Nde I + Kpn I
Kpn I
pBW7.01
=(Gly4 Ser)3
LINKER
Kpn I
pBW7.0

FIG. 36

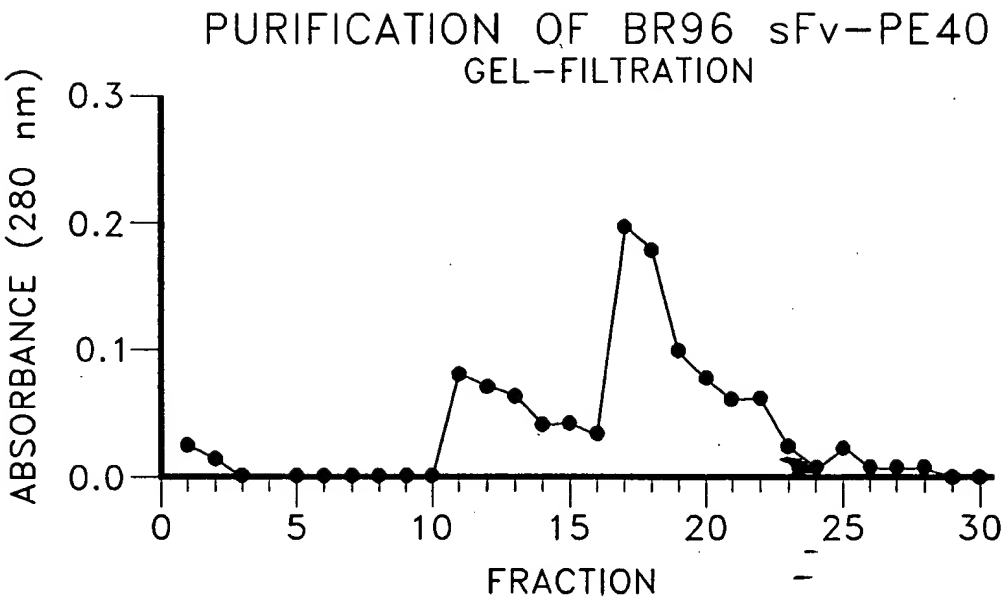

PURIFICATION OF BR96 sFv-PE40
GEL-FILTRATION
ABSORBANCE (280 nm)
0.3
0.2
0.1
0.0
0
5
10
15
20
25
30
FRACTION

FIG. 37A

Figure 37 B.

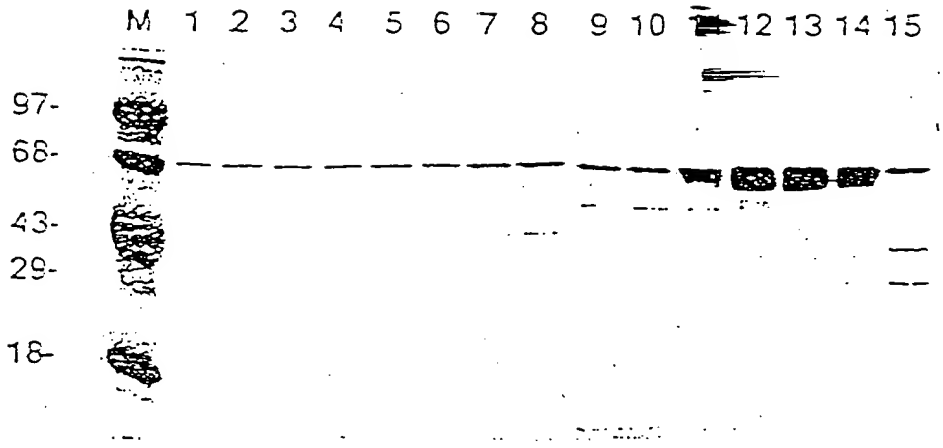
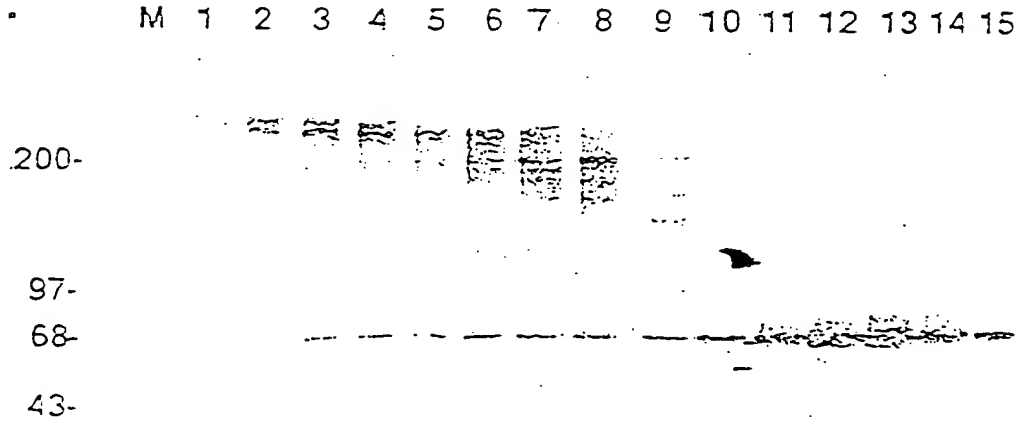


Figure 37C.



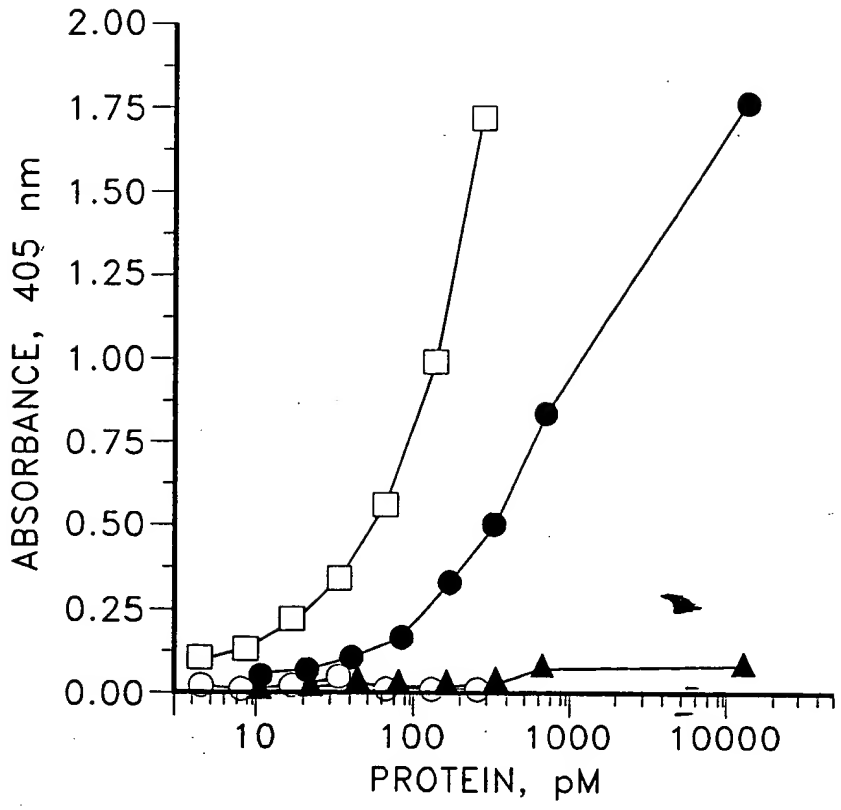

2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
ABSORBANCE, 405 nm
10
100
1000
10000
PROTEIN, pM

FIG. 38

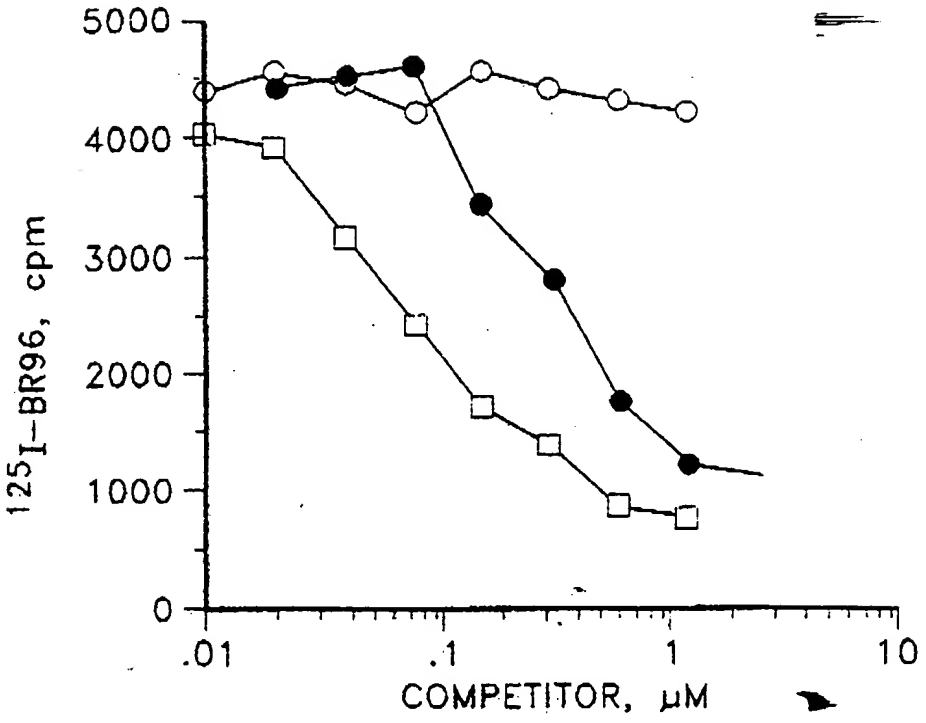

5000
4000
3000
2000
1000
0
^{125}I-BR96, cpm
.01
.1
1
10
COMPETITOR, μM

FIG. 39

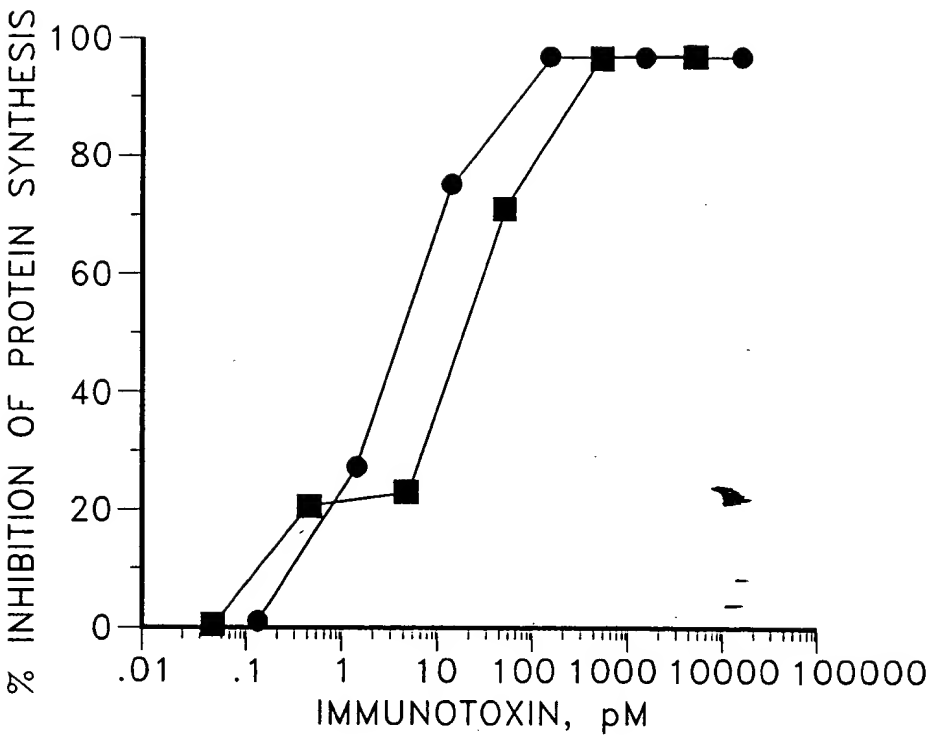

% INHIBITION OF PROTEIN SYNTHESIS
100
80
60
40
20
0
.01
.1
1
10
100
1000
10000
100000
IMMUNOTOXIN, pM

FIG. 40

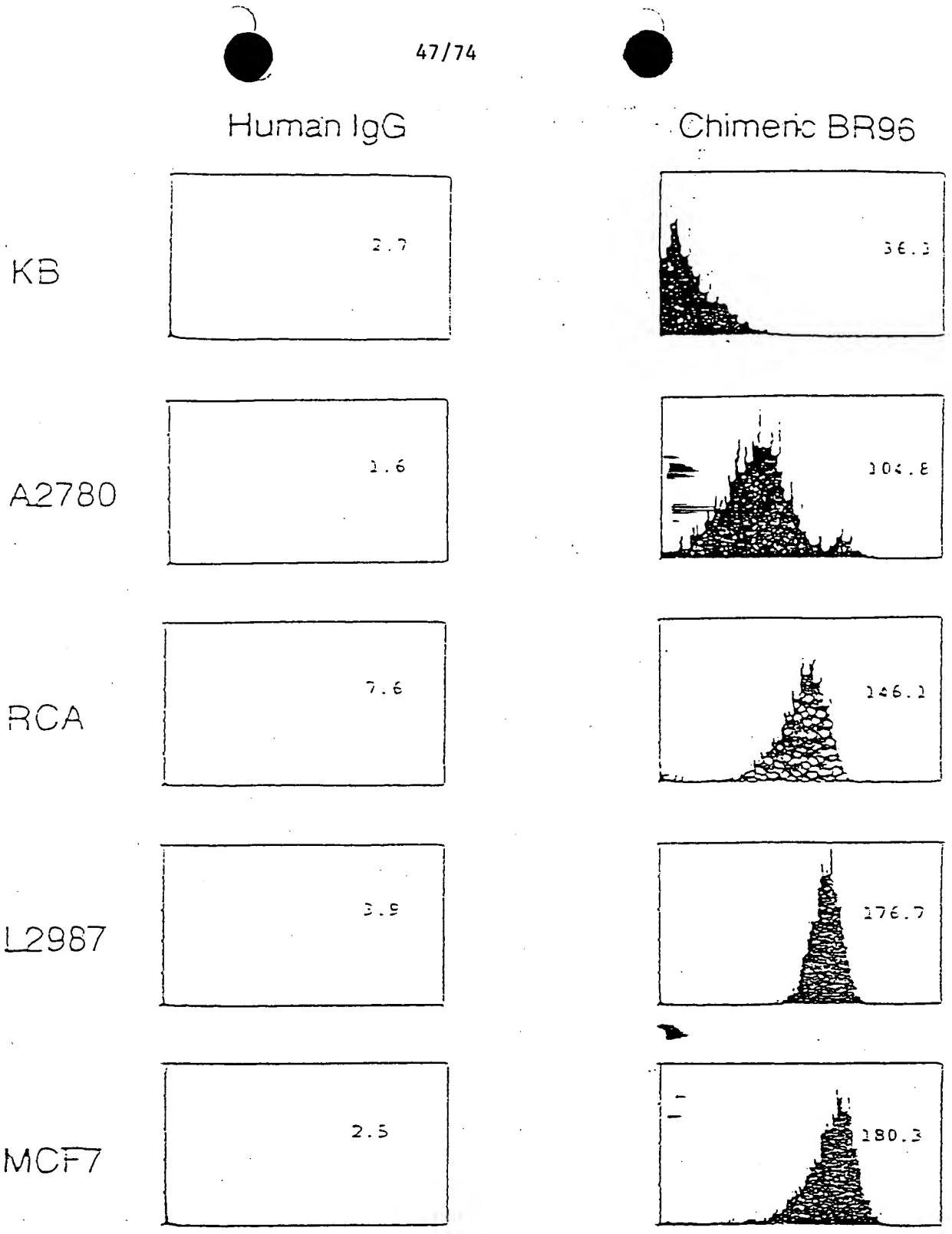

Human IgG
Chimeric BR96
KB
2.7
36.3
A2780
1.6
104.8
RCA
7.6
146.1
L2987
3.9
176.7
MCF7
2.5
180.3

FIGURE 41

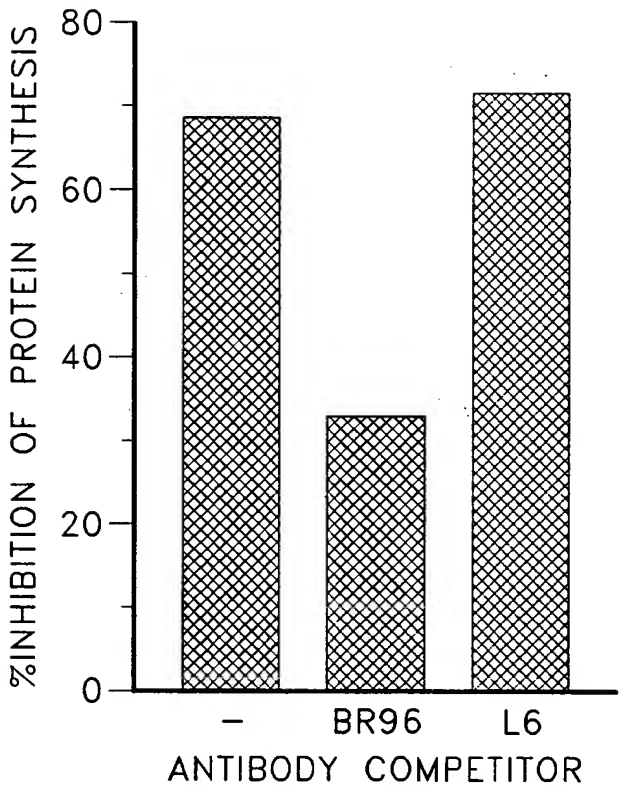

%INHIBITION OF PROTEIN SYNTHESIS
80
60
40
20
0
–
BR96
L6
ANTIBODY COMPETITOR

FIG. 42

MCF7 HUMAN BREAST TUMOR XENOGRAFT
BR96 sFv–PE40 TREATMENT STARTED ON d12

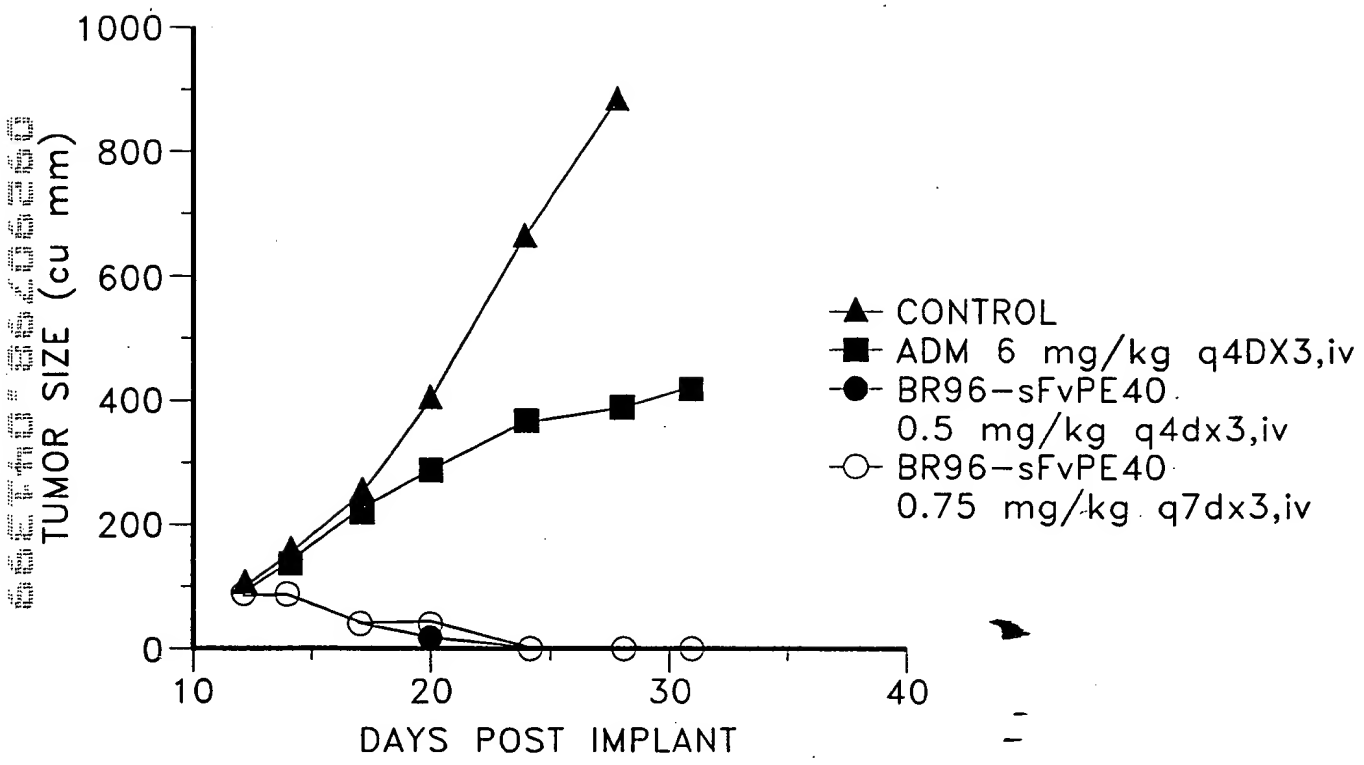


FIG. 43

ANTI-TUMOR ACTIVITY OF BR96-IMMUNOTOXINS AGAINST L2987 HUMAN LUNG TUMOR XENOGRAFTS

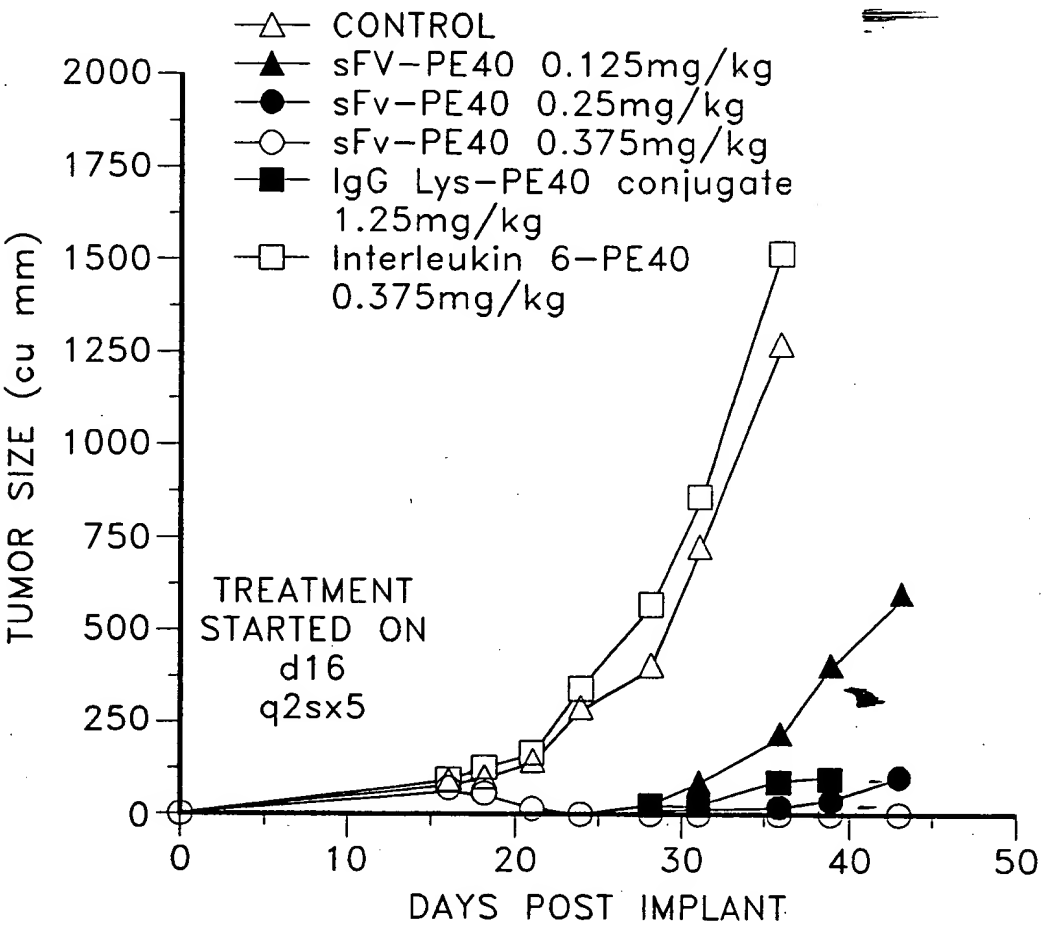


FIG. 44

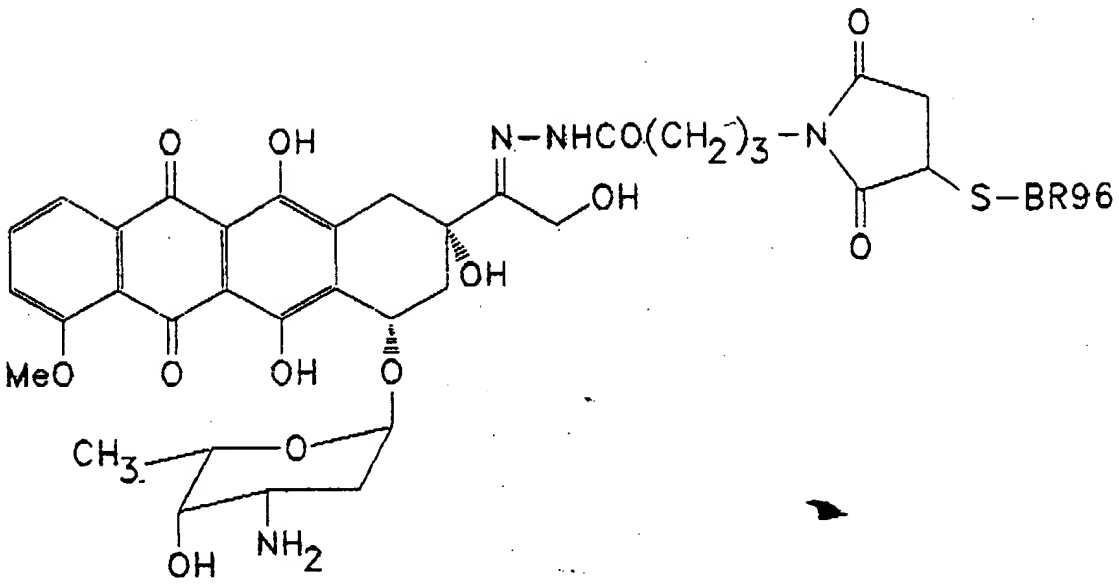


FIG. 45

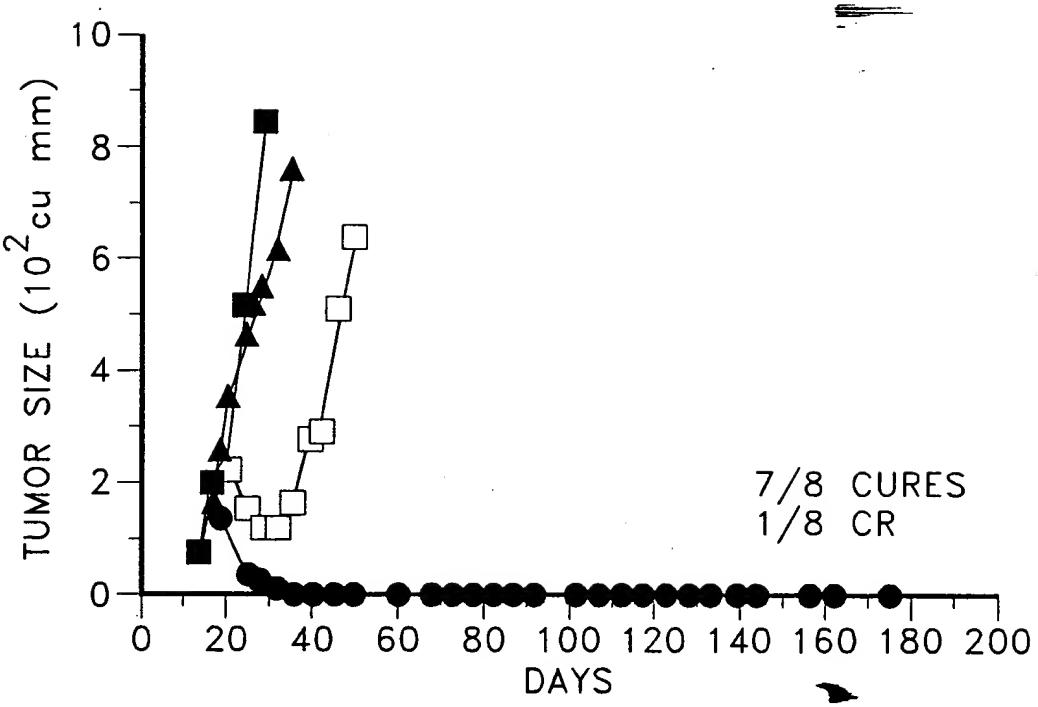

TUMOR SIZE (10^2cu mm)
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
180
200
DAYS
7/8 CURES
1/8 CR

FIG. 46A

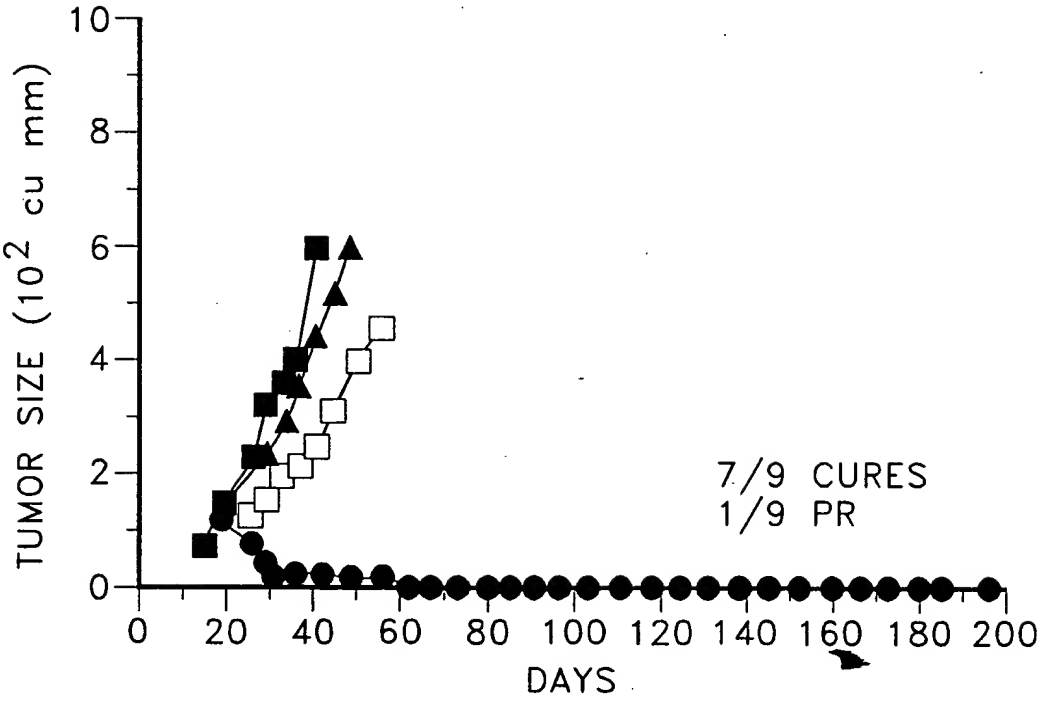

TUMOR SIZE (10^2 cu mm)
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
180
200
DAYS
7/9 CURES
1/9 PR

FIG. 46B

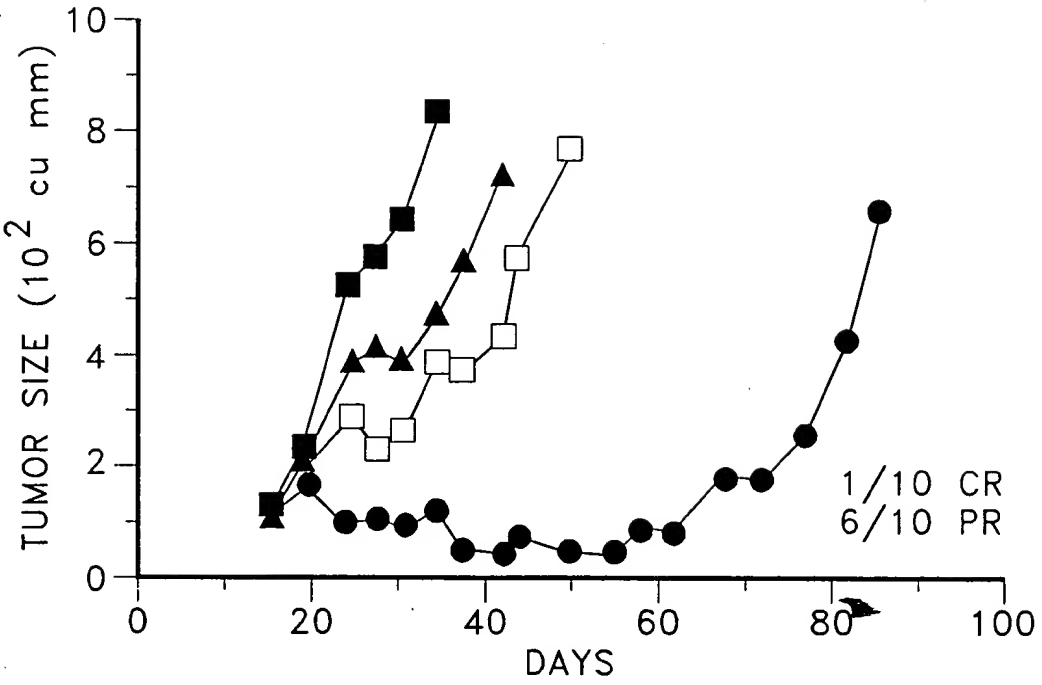


FIG. 46C

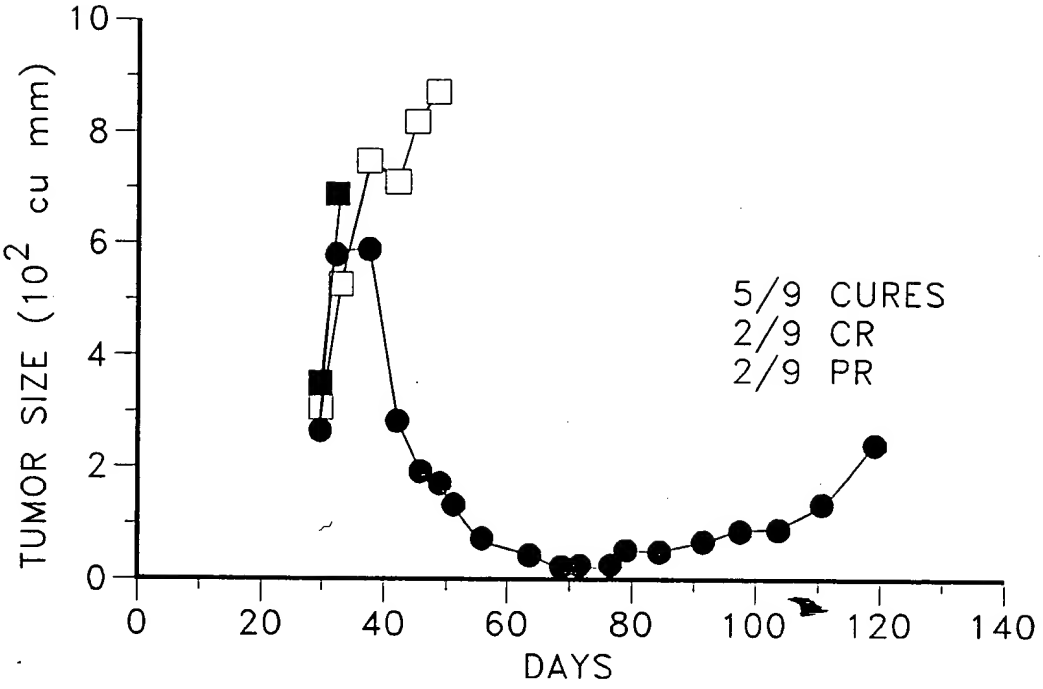

TUMOR SIZE (10^2 cu mm)
10
8
6
4
2
0
0
20
40
60
80
100
120
140
DAYS
5/9 CURES
2/9 CR
2/9 PR

FIG. 46D

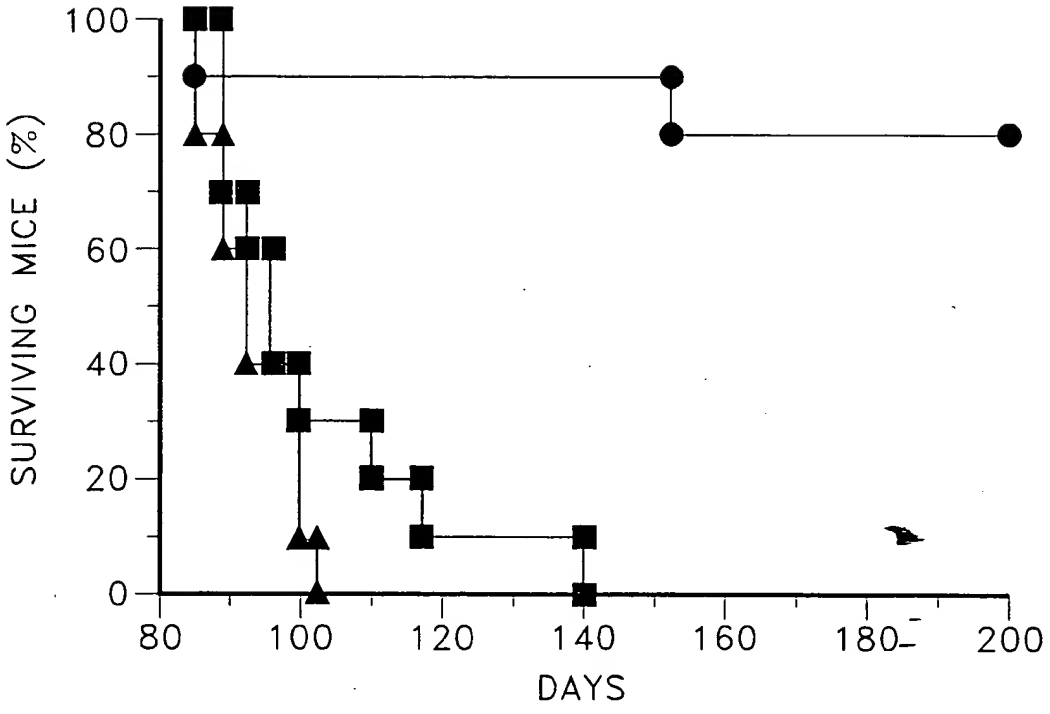


FIG. 47

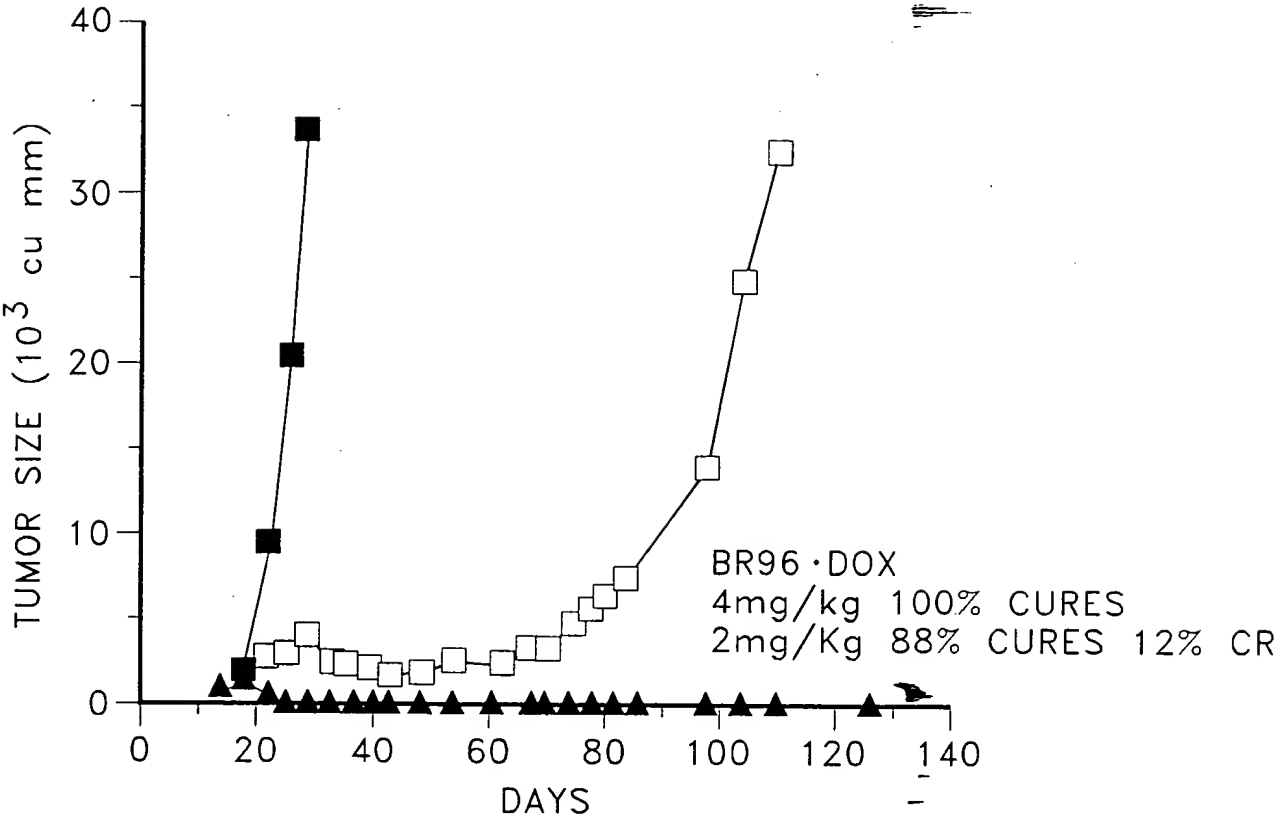
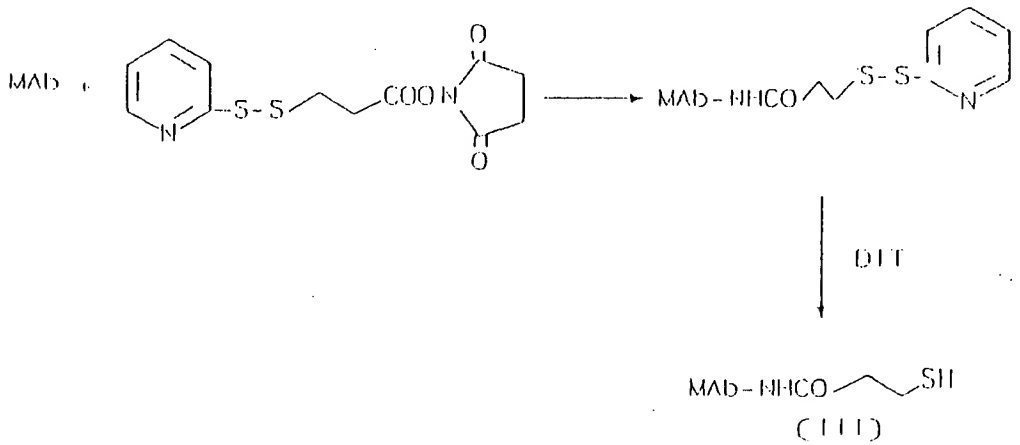

TUMOR SIZE (10^3 cu mm)
40
30
20
10
0
0
20
40
60
80
100
120
140
DAYS
BR96·DOX
4mg/kg 100% CURES
2mg/Kg 88% CURES 12% CR

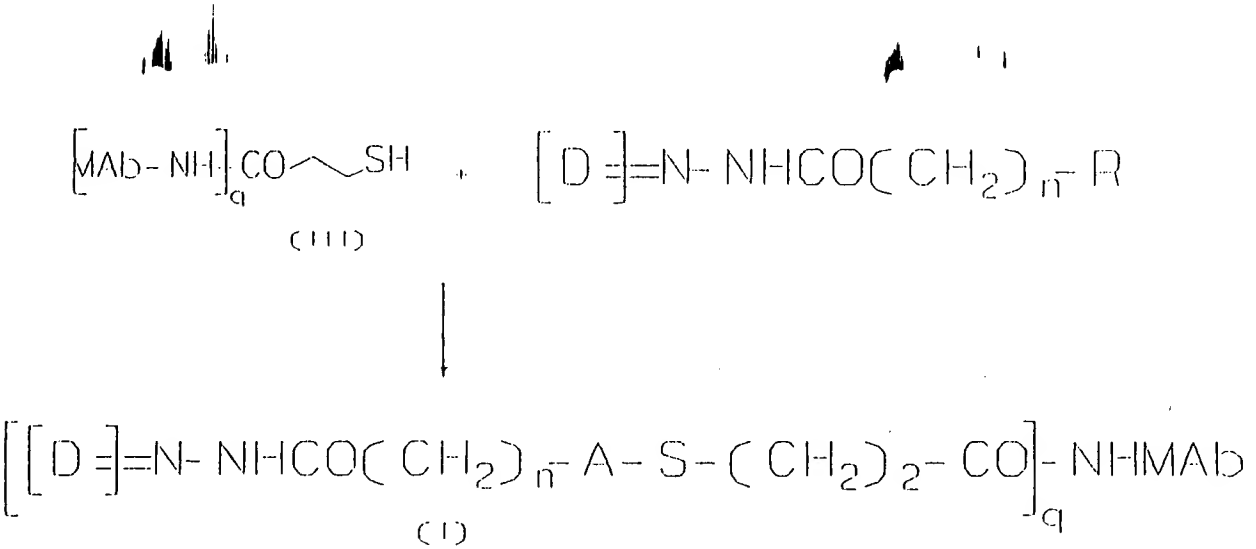
FIG. 48

FIGURE 49a



(III)

FIGURE 49b



0925079B_D41399

FIGURE 49c

MAb
S-A-(CH2)n-CONH-N=[D]
(I)
MAb
S-A-(CH2)n-CONH-N=[D]
SH
RELAXED MAb
+
R-n(CH2)COONH-N=[D]
(IIa)
DTT
MAb
S
S

FIGURE 50

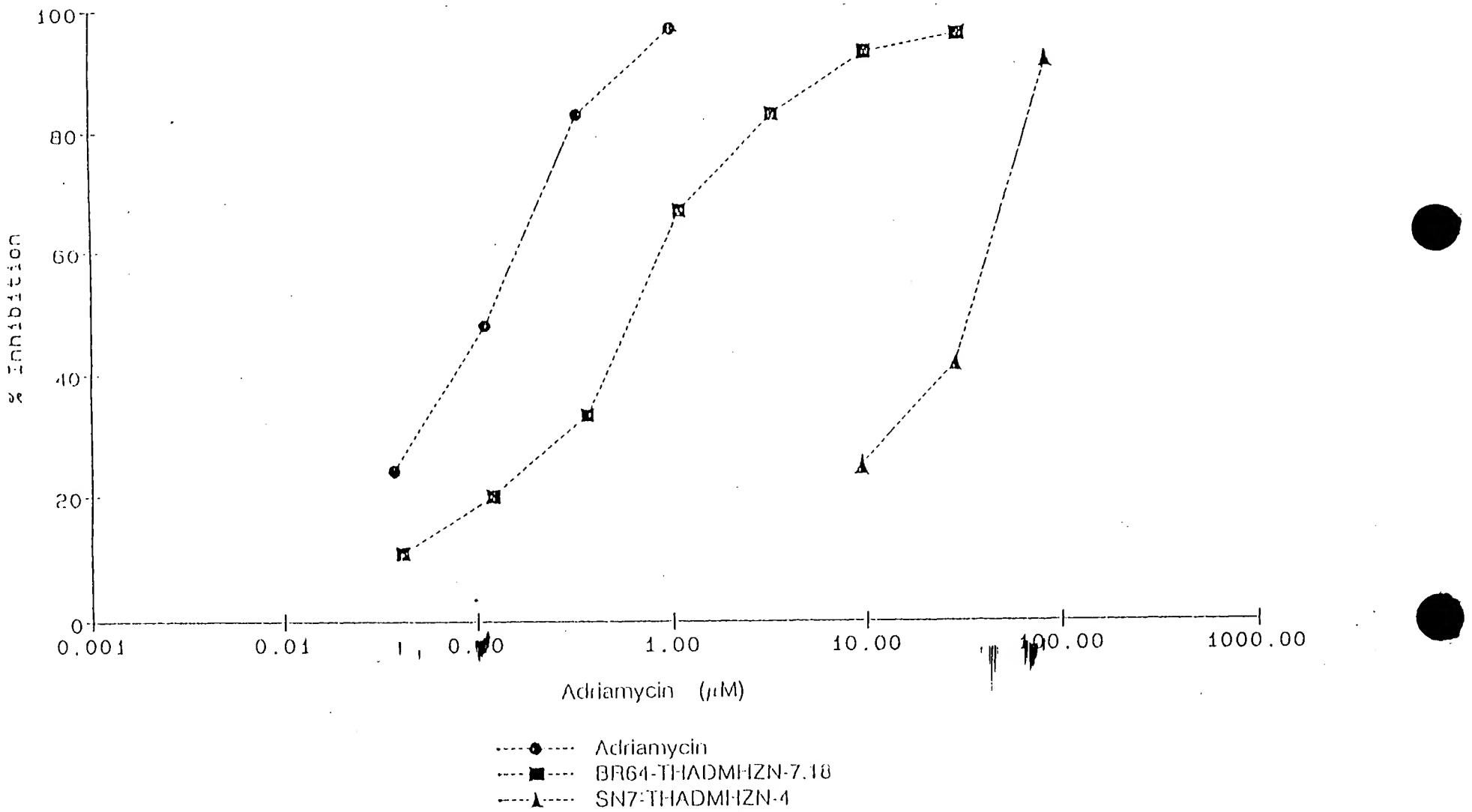

100
80
60
40
20
0
% Inhibition
0.001
0.01
0.10
1.00
10.00
100.00
1000.00
Adriamycin (μM)
Adriamycin
BR64-THADMHZN-7.18
SN7-THADMHZN-4

FIGURE 52

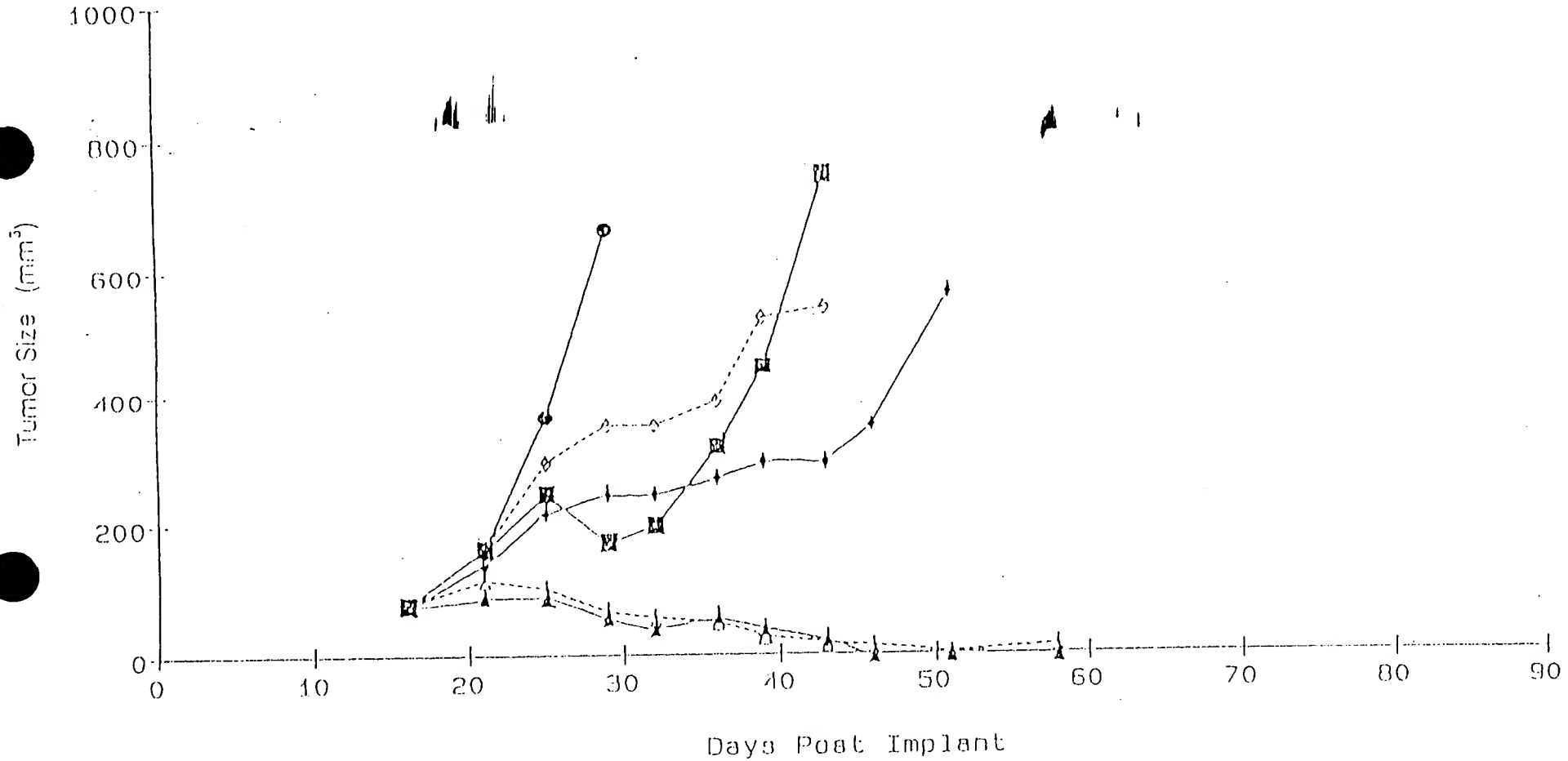
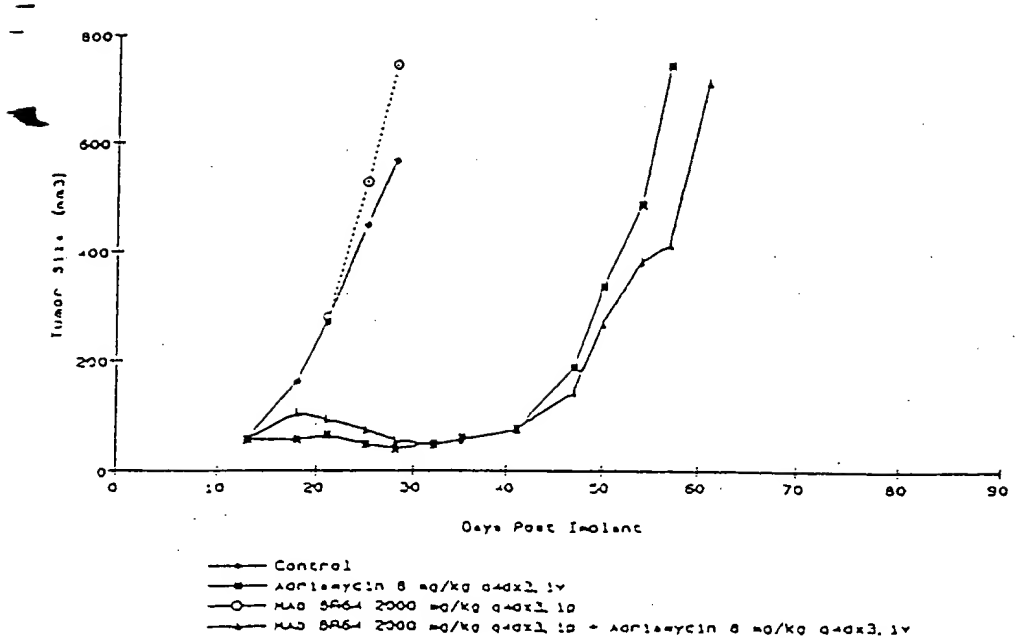

1000
800
600
400
200
0
Tumor Size (mm³)
0
10
20
30
40
50
60
70
80
90
Days Post Implant
Control
Adriamycin 8 mg/kg q4dx3, iv
BR64-THADNHZN-7.18 15 mg/kg adria 583 mg/kg BR64 q4dx3, ip
BR64-THADMHZN-7.18 10 mg/kg adria 389 mg/kg BR64 q4dx3, ip
SN7-THADMHZN-4 15 mg/kg adria 1020 mg/kg SN7 q4dx3, ip
SN7-THADMHZN-4 10 mg/kg adria 680 mg/kg SN7 q4dx3, ip

FIGURE 53 a/b

(a)



(b)

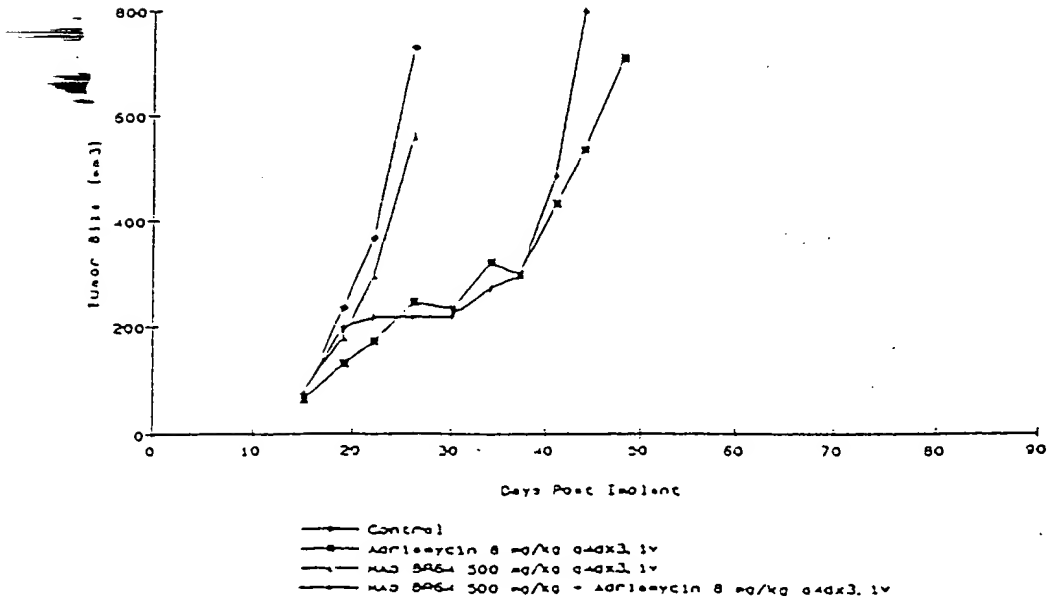


FIGURE 53 (c)

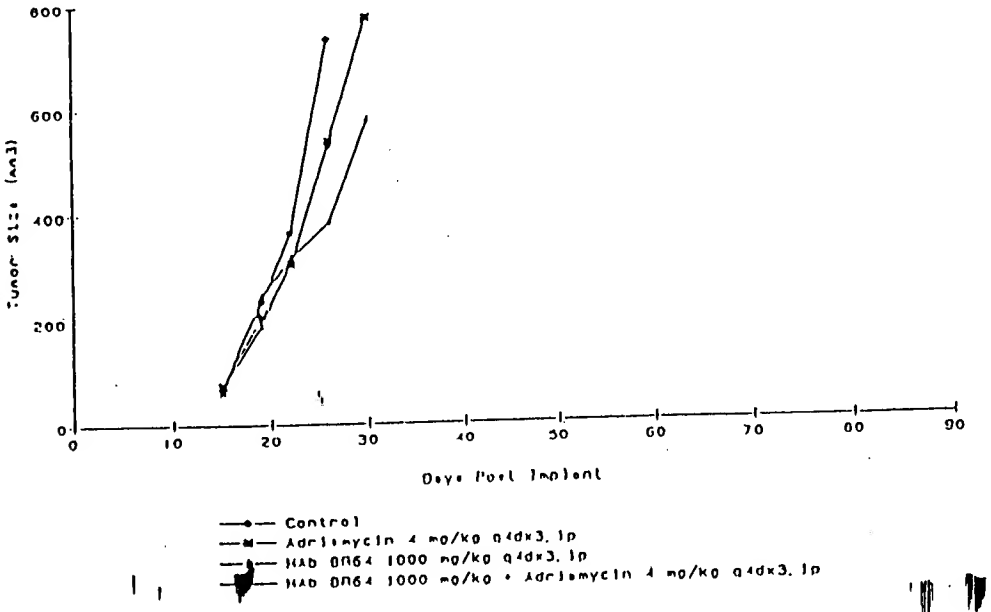

600
600
400
200
0
0
10
20
30
40
50
60
70
80
90
Days Post Implant
Control
Adriamycin 4 mg/kg q4dx3, ip
MAb BR64 1000 mg/kg q4dx3, ip
MAb BR64 1000 mg/kg + Adriamycin 4 mg/kg q4dx3, ip

FIGURE 54

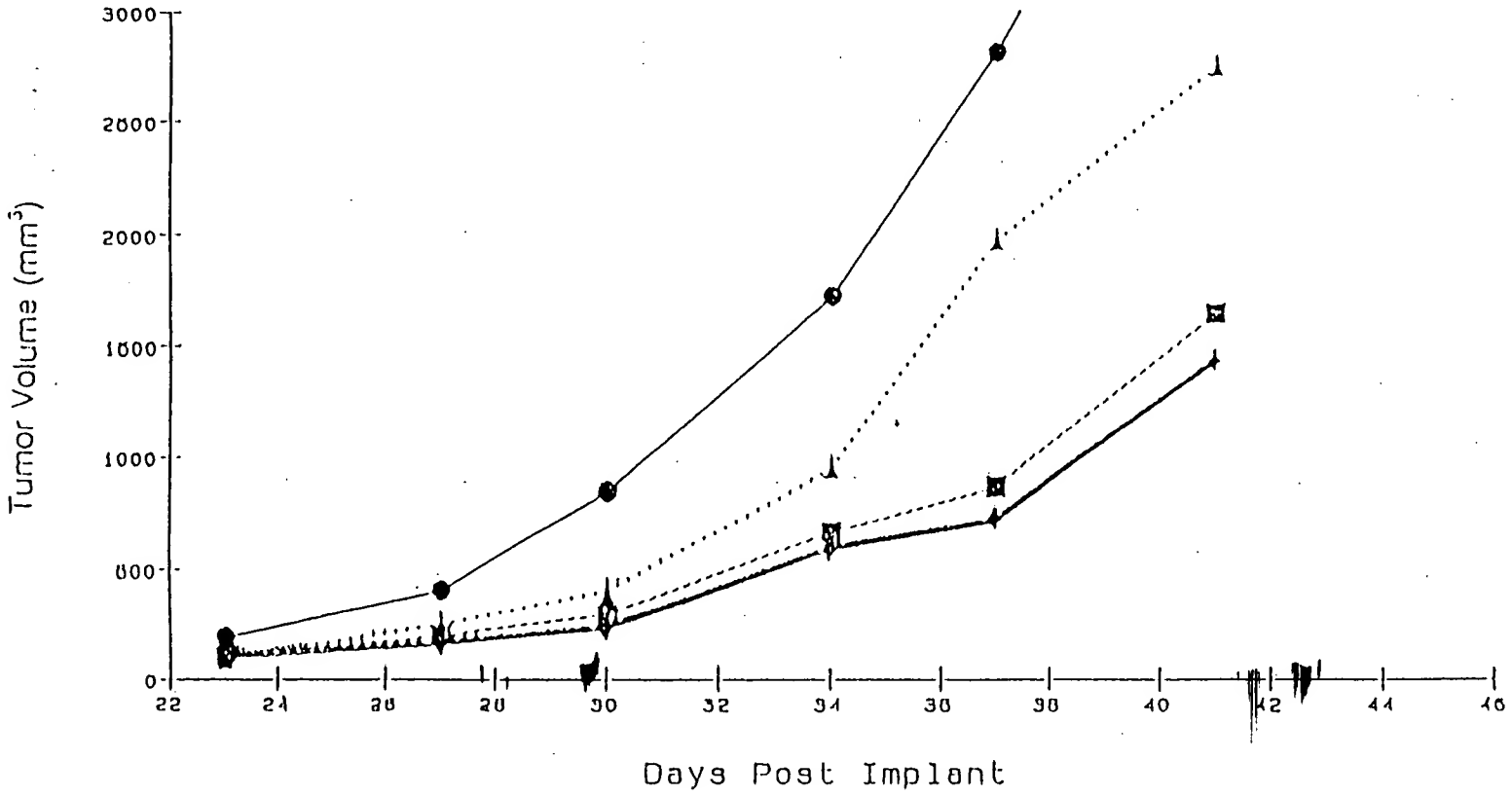

Tumor Volume (mm^3)
3000
2000
1000
0
22
24
26
28
30
32
34
36
38
40
42
44
46
Days Post Implant
Control
ADM
1.6 MG/KG ADM IV Q2DX5
P77-ADM(TH)
1.6 MG/KG ADM IV Q2DX5
BN-ADM(TH)
1.6 MG/KG ADM IV Q2DX5

FIGURE 55

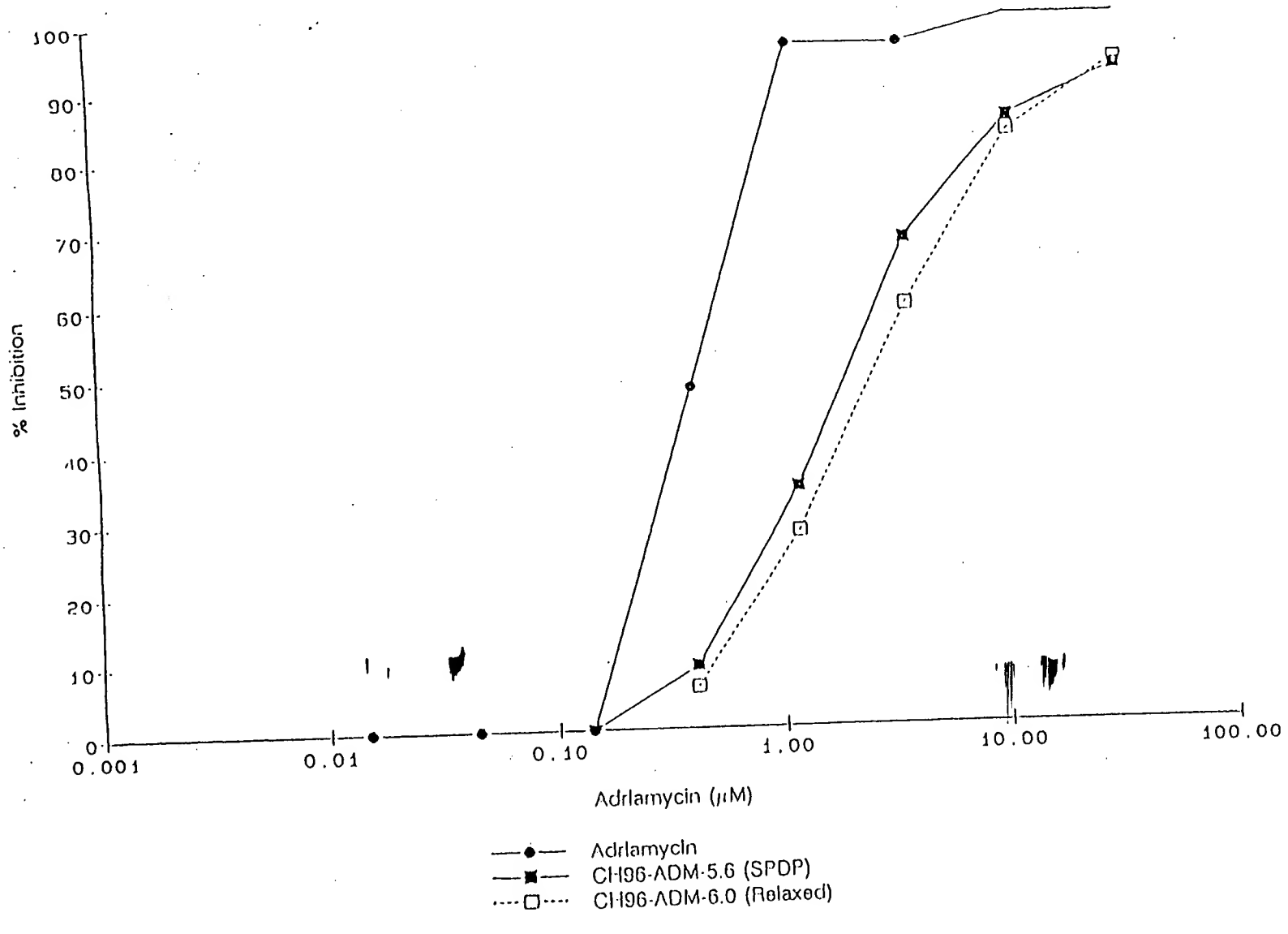
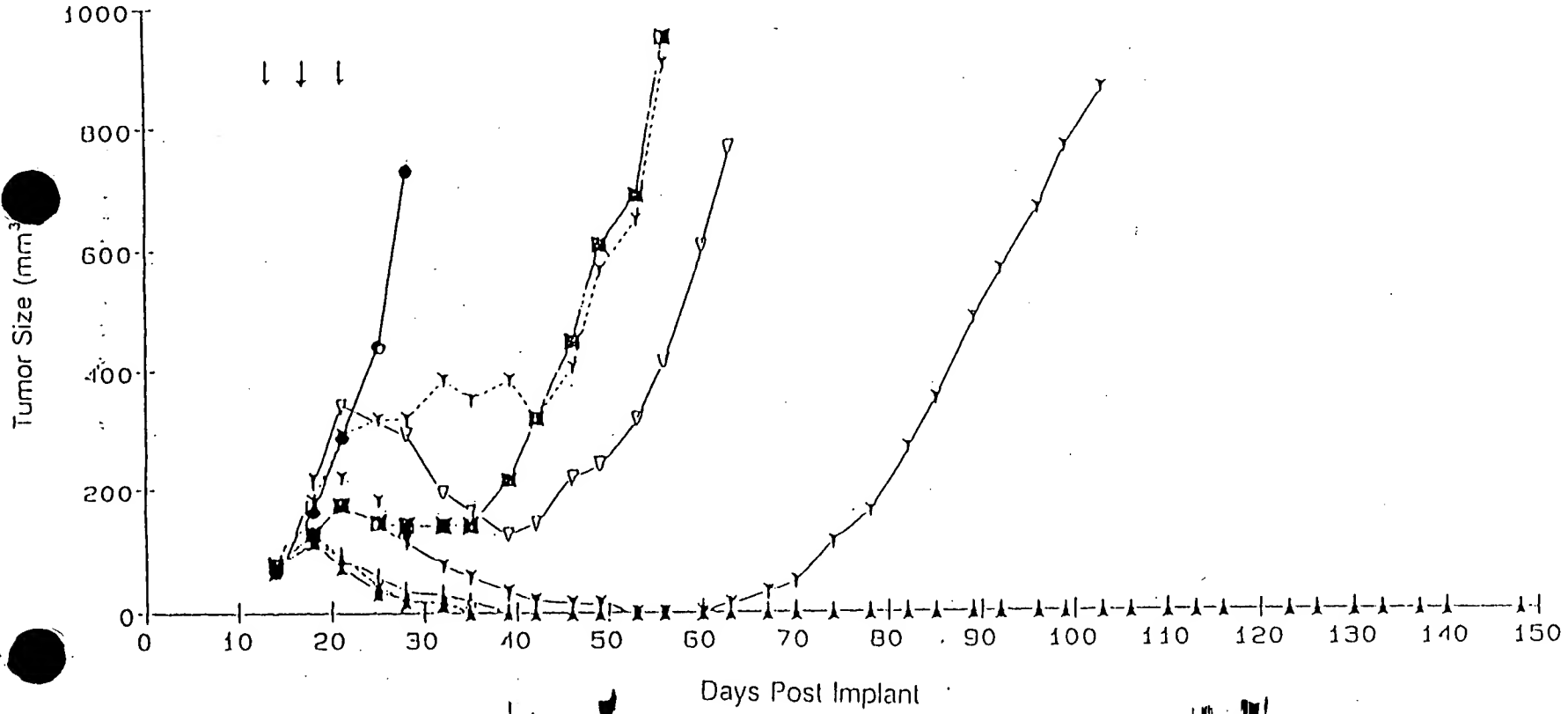

100
90
80
70
60
50
40
30
20
10
0
% Inhibition
0.001
0.01
0.10
1.00
10.00
100.00
Adriamycin (μM)
Adriamycin
CH96-ADM-5.6 (SPDP)
CH96-ADM-6.0 (Relaxed)

FIGURE 56



0920798 .04.1399

FIGURE 57

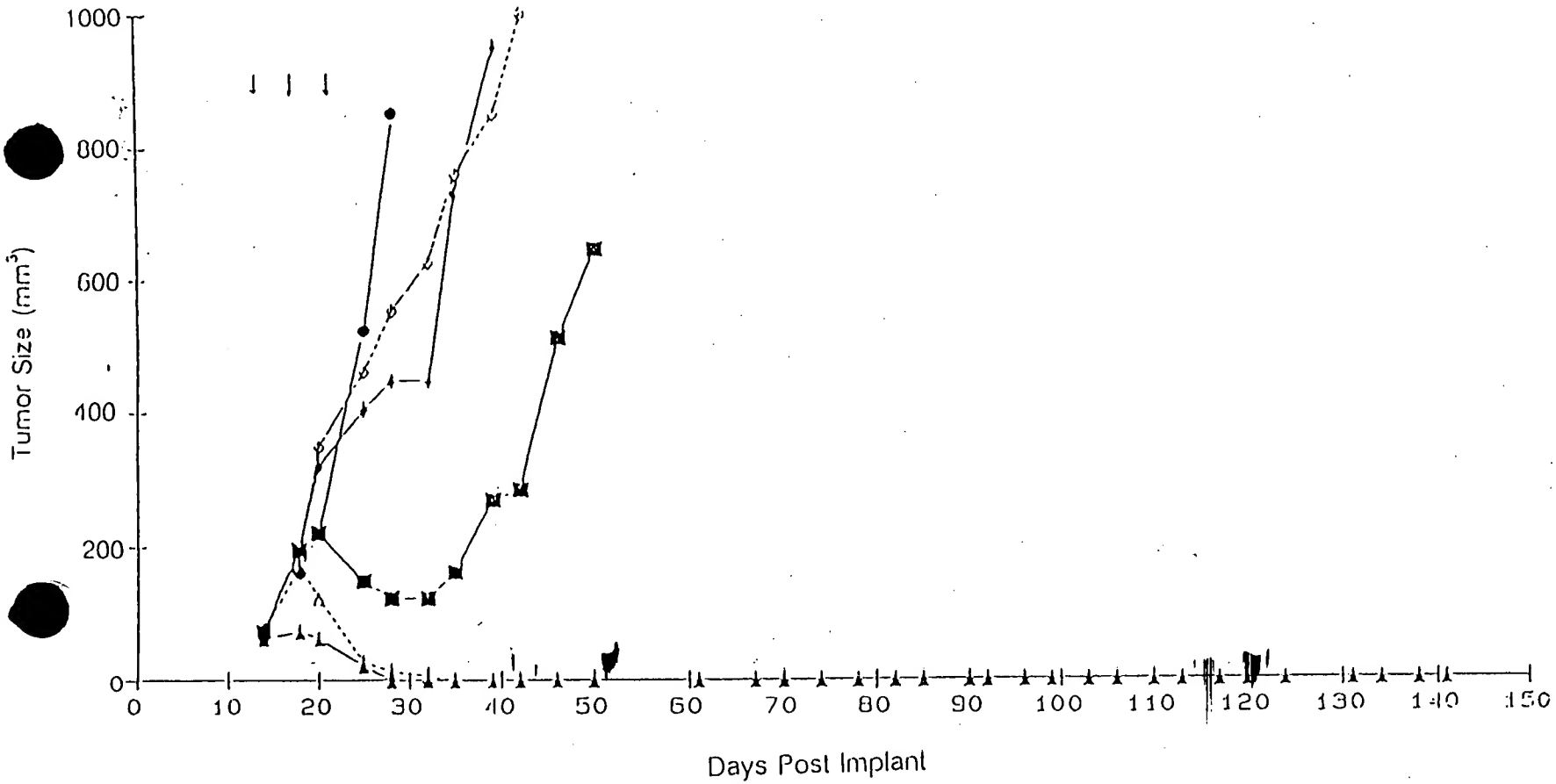

1000
800
600
400
200
0
Tumor Size (mm³)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Days Post Implant
Control
Adriamycin 8 mg/kg, iv
ChiBR96-ADM-4.19 10 mg/kg ADM 666 mg/kg ChiBR96, ip
ChiBR96-ADM-4.19 5 mg/kg ADM 333 mg/kg ChiBR96, ip
IgG-ADM-7.16 10 mg/kg ADM 226 mg/kg IgG, ip
IgG-ADM-7.16 5 mg/kg ADM 113 mg/kg IgG, ip

FIGURE 58

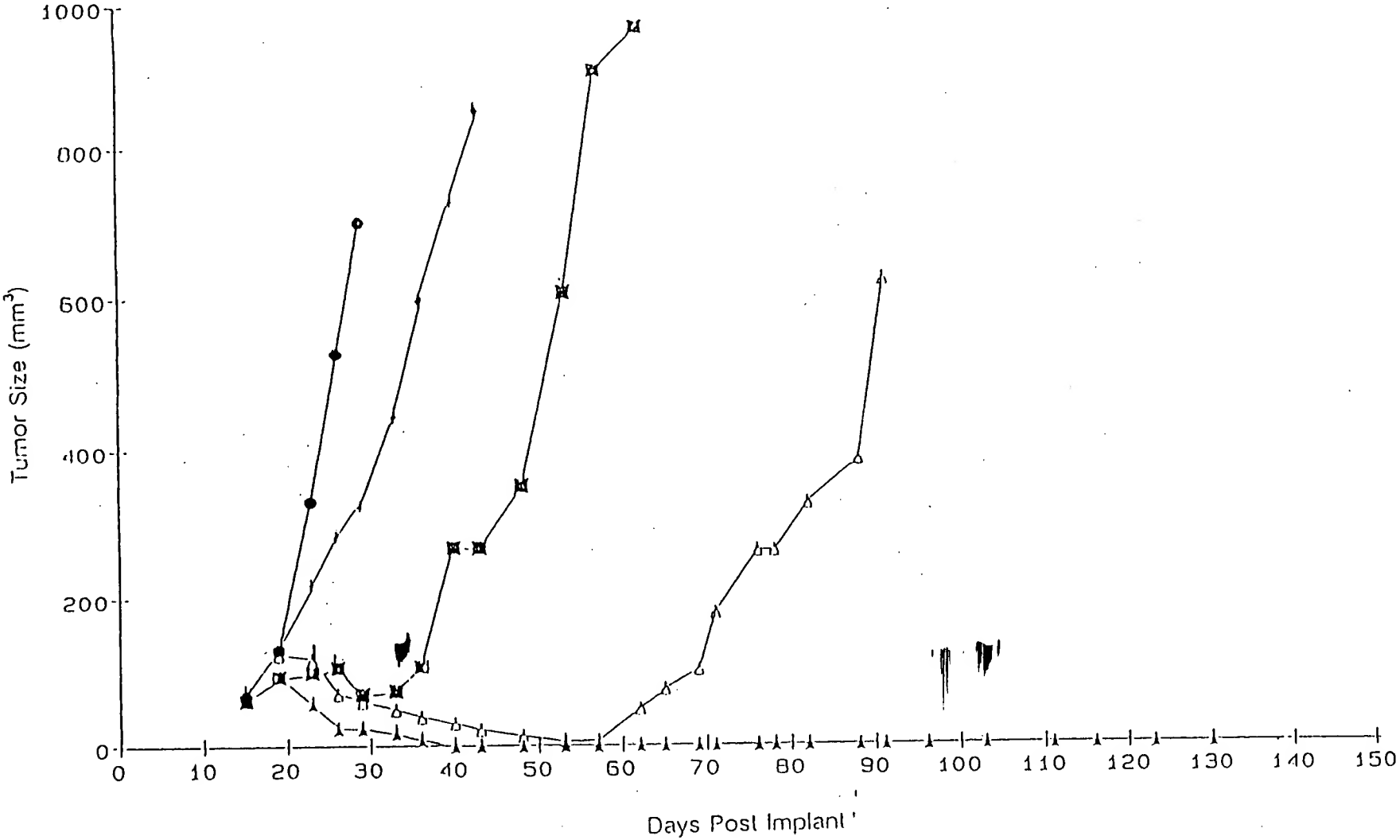

1000
000
600
400
200
0
Tumor Size (mm³)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Days Post Implant
Control
Adriamycin
8 mg/kg q1dx3, iv
ChiBR96-ADM-5.80
10 mg/kg ADM
500 mg/kg ChiBR96 q1dx3, ip
ChiBR96-ADM-5.80
5 mg/kg ADM
250 mg/kg ChiBR96 q1dx3, ip
IgG-ADM-7.16
10 mg/kg ADM
380 mg/kg q1dx3, ip

FIGURE 59

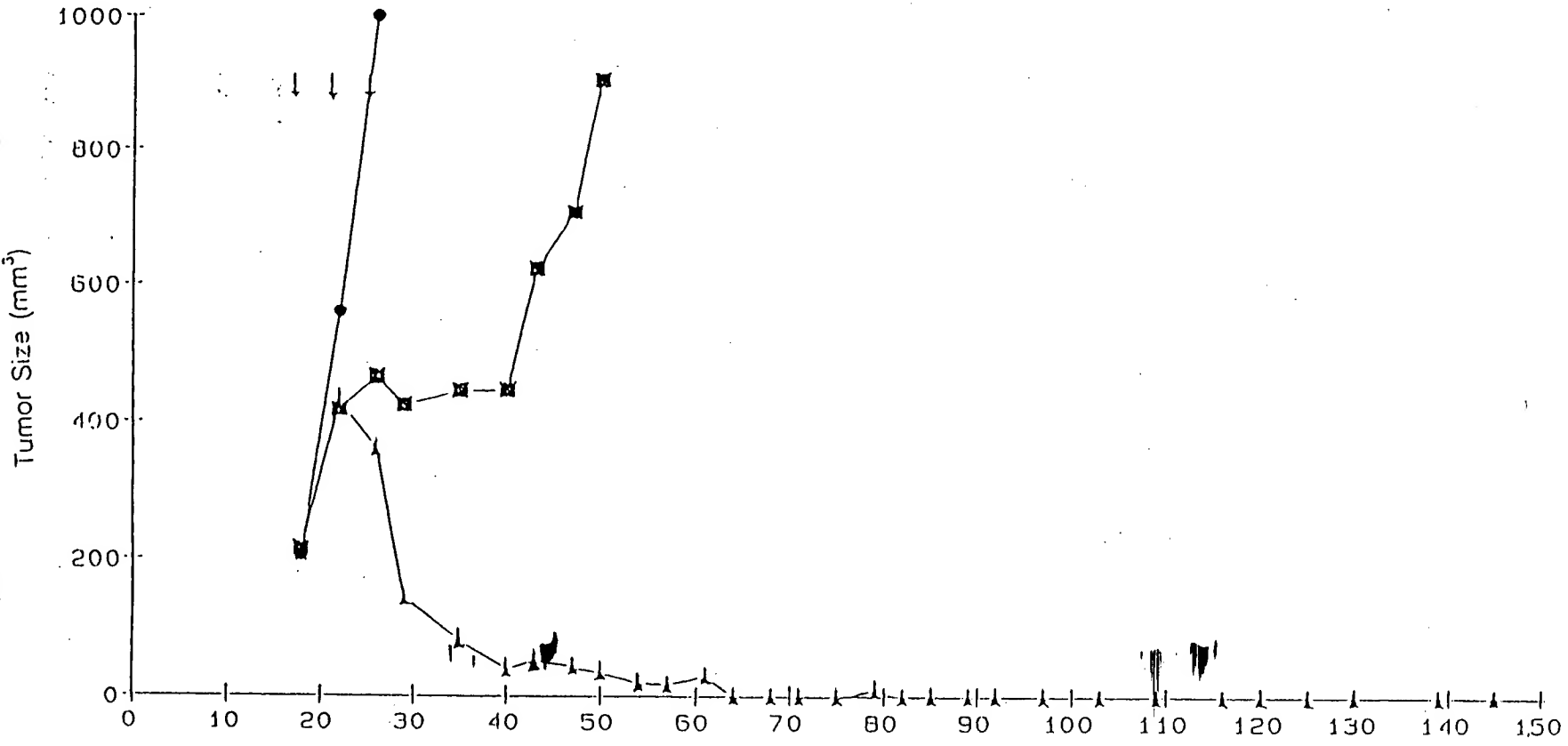

1000
800
600
400
200
0
Tumor Size (mm^3)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Days Post Implant
Control
Adriamycin
8 mg/kg, iv
ChiBR96-ADM-6.85
10 mg/kg, ip

FIGURE 60

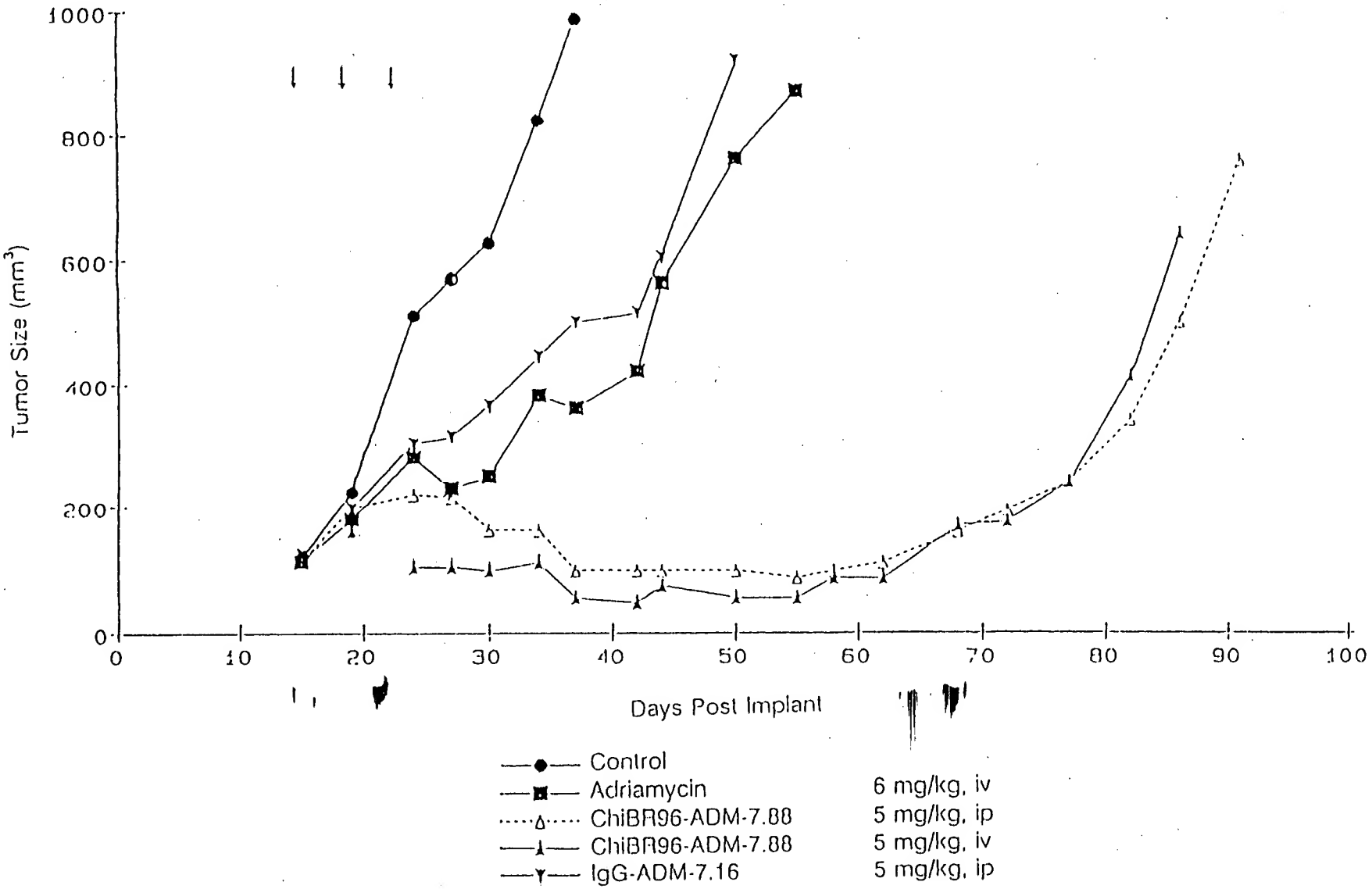

1000
800
600
400
200
0
Tumor Size (mm³)
0
10
20
30
40
50
60
70
80
90
100
Days Post Implant
Control
Adriamycin
ChiBR96-ADM-7.88
ChiBR96-ADM-7.88
IgG-ADM-7.16
6 mg/kg, iv
5 mg/kg, ip
5 mg/kg, iv
5 mg/kg, ip

FIGURE 61

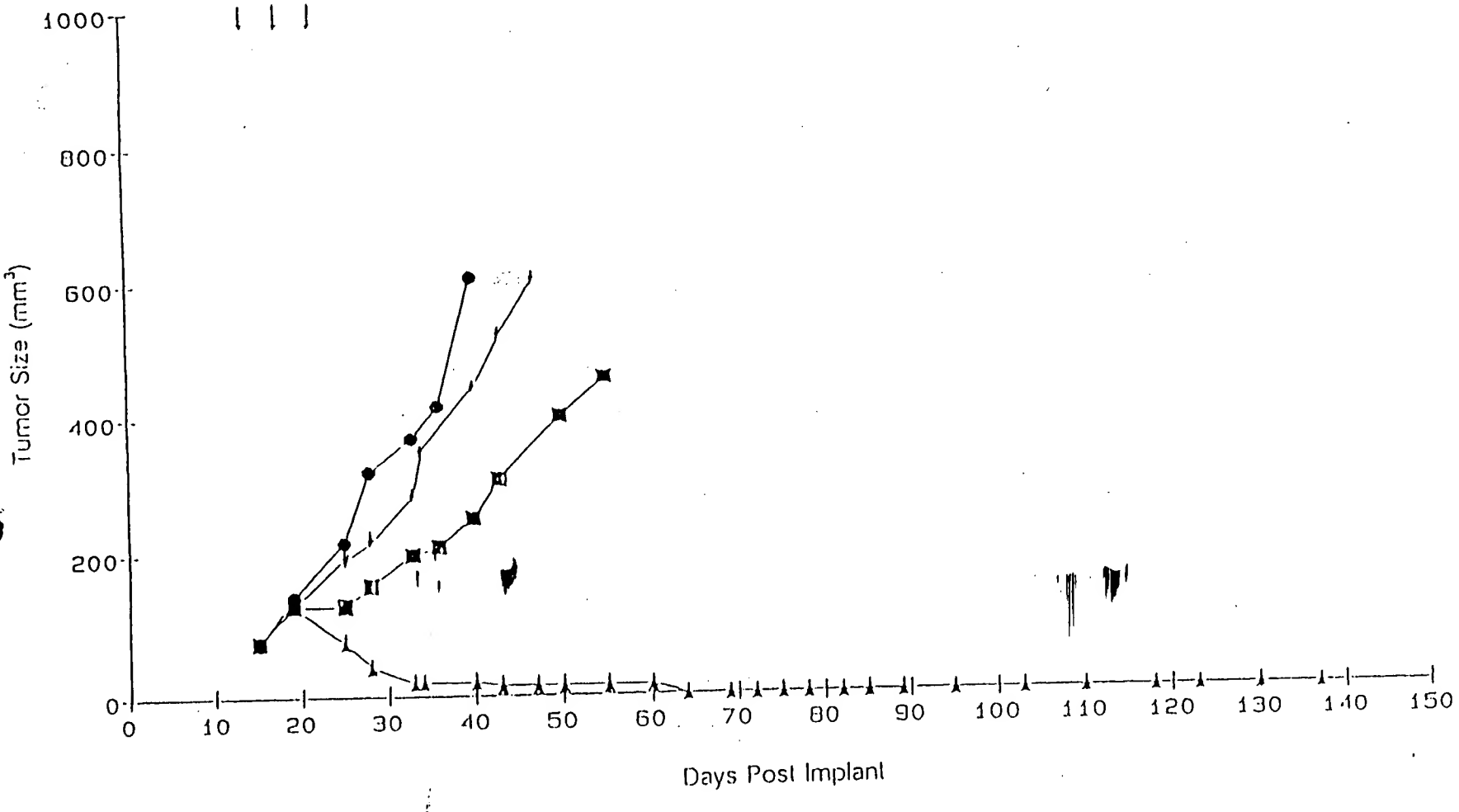
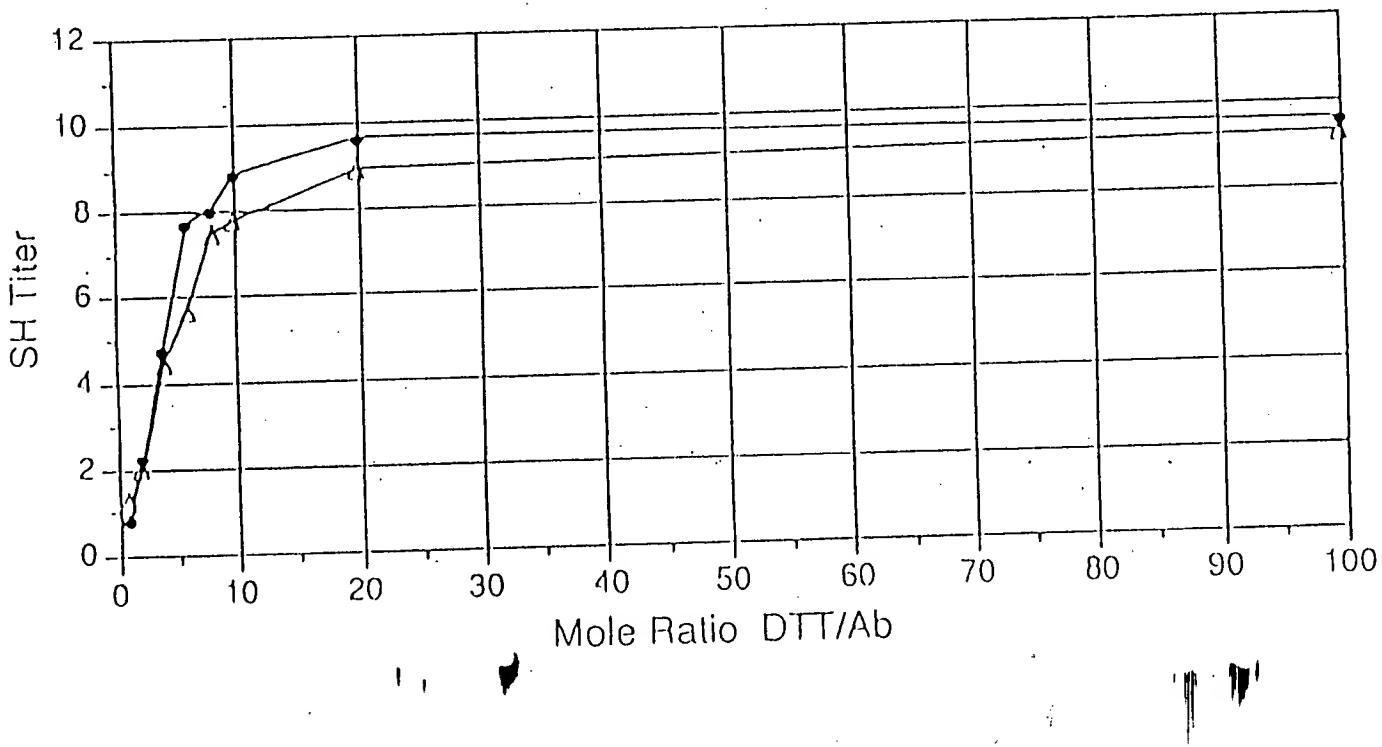

1000
800
600
400
200
0
Tumor Size (mm³)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Days Post Implant
Control
Adriamycin
ChiBR96-ADM-7.80
IgG-ADM-7.16
8 mg/kg, q4dx3, iv
10 mg/kg ADM 350 mg/kg ChiBR96, ip
10 mg/kg ADM 400 mg/kg IgG, ip

FIGURE 62



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